UNIVERSITY OF CALIFORNIA
MAY 13 1947

American Journal of Veterinary Research

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A Publication of the American Veterinary Medical Association

Volume VIII APRIL, 1947 Number 27

Consecutive order of appearance, rather than identity with any given volume, is the plan adopted for numbering the issues of the American Journal of Veterinary Research. Following are the volume and issue numbers published to date.

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VOL. II, No. 2—JANUARY, 1941
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VOL. VII, No. 23—APRIL, 1946 (PART 2)
VOL. VII, No. 24—JULY, 1946
VOL. VII, No. 25—OCTOBER, 1946

VOL. VIII, No. 26—JANUARY, 1947
VOL. VIII, No. 27—APRIL, 1947

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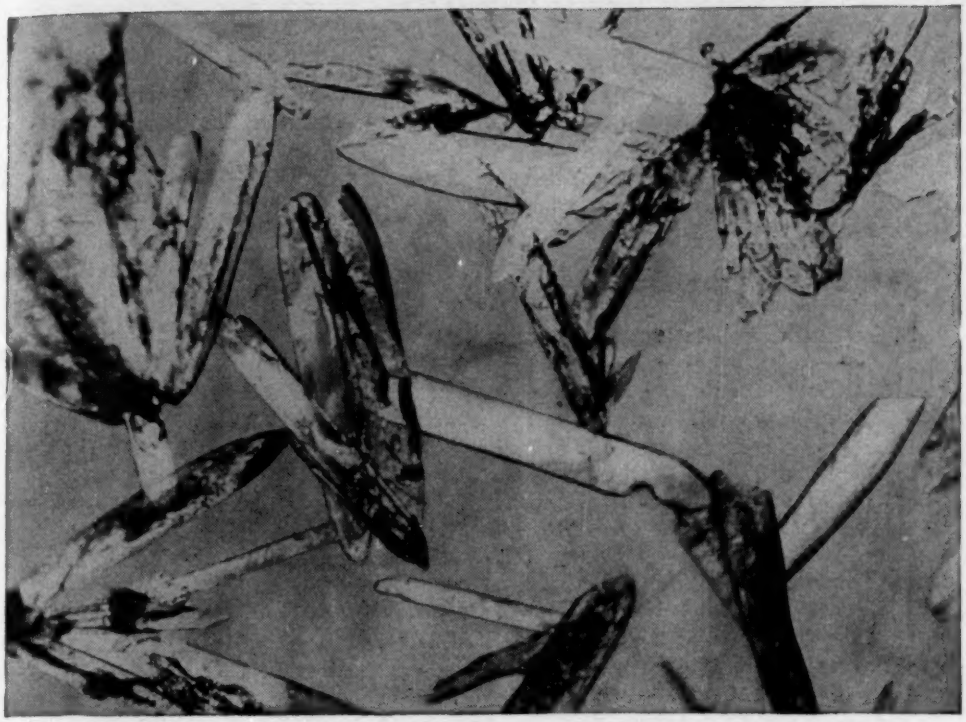
Published quarterly at 600 S. Michigan Ave., Chicago, Ill., by the American Veterinary Medical Association. Entered as Second-Class Matter January 31, 1941, at the Post Office at Chicago, Illinois, under the Act of March 3, 1879. Subscription price: $4.00 per year for United States, Canada, and countries affiliated with the Pan American Postal Union; foreign countries, $4.50. Members of the American Veterinary Medical Association may subscribe to the American Journal of Veterinary Research for $2.50 per year added to the annual dues of $7.00. Single copies $1.25. Address all correspondence to American Veterinary Medical Association.

Printed in the U. S. A.



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VOL. VIII APRIL, 1947 No. 27

The Blood in Equine Periodic Ophthalmia

CAPTAIN T. O. ROBY, V.C. and MAJOR T. C. JONES, V.C.
Fort Robinson, Nebraska

THE LESIONS of equine periodic ophthalmia are localized in the eye, but it seems highly possible that they may be linked with other systemic irregularities. We have observed that a slight and transient temperature rise often occurs in the early part of the acute stages of periodic ophthalmia. In an unrelated study, Earle[1] noted significant changes in the glucose tolerance of a horse which was affected with periodic ophthalmia. It was largely due to the suggestion of Dr. Earle that glucose tolerance tests were included in our experiments. Campori *et al*,[2] found no variation from normal in chemical analyses of blood from horses with periodic ophthalmia. However, this was not an exhaustive study. The recognition of any generalized metabolic disturbance is essential to a complete understanding of the disease. These studies were undertaken to learn if any deviation from normal occurred in the blood of horses affected with periodic ophthalmia.

Quantitative chemical and hematologic determinations were made on the principal blood constituents of horses in the quiescent and acute stages of the disease.* The criteria for classification of cases of periodic ophthalmia into acute and quiescent stages has been described by Jones[3] in an earlier work. The term *periodic ophthalmia*, used throughout this paper, designates more specifically equine recurrent iridocyclitis. All of the horses, previous to their use in this study, had exhibited two or more acute attacks of iridocyclitis.

TABLE I—Average Blood Chemistry Values of Horses with Acute and Quiescent Iridocyclitis and Horses with Normal Eyes

	Determination	Horses with acute iridocyclitis	Horses with quiescent iridocyclitis	Horses with normal eyes
Mgm. per 100 cc of whole blood	Non-Protein Nitrogen	35.2	33.9	37.2
	Urea	29.7	25.9	30.1
	Creatinine	1.71	1.58	1.8
	Uric Acid	1.77	1.66	1.88
	Glucose	80.0	75.1	77.3
	Chlorides (as NaCl)	479.6	437.5	449.5
	Cholesterol	137.0	143.1	132.0
Mgm. per 100 c.c. of serum	Inorganic Phosphorus	4.21	3.3	3.8
	Calcium	12.18	11.54	12.38
	Bilirubin	2.40	2.64	2.17
	Potassium	15.93	18.19	17.09
Units	Icterus Index	11.8	13.6	10.2
Gms. per 100 cc plasma	Total Serum Protein	7.19	7.04	6.98
	Serum Albumin	3.19	3.706	3.77
	Serum Globulin	4.00	3.334	3.21
	Fibrinogen	.85	.78	.84
Units	Phosphatase	11.27	11.22	10.34

The blood from a group of healthy animals with no ocular disease was examined by the same procedures and used to establish normal values for the horse. The following determinations were made: nonprotein nitrogen, urea, creatinine, uric acid, glucose,

Presented before the Section on Research, Eighty-third Annual Meeting, Boston, Mass., Aug. 18-22, 1946.

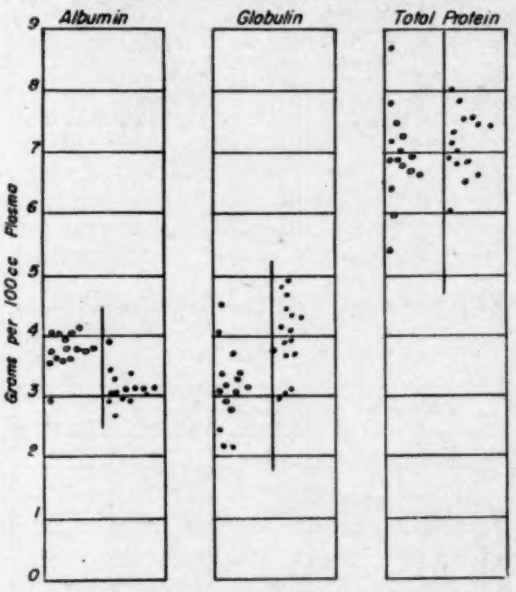
From the Veterinary Research Laboratory, Fort Robinson, Neb. (Roby); from the Army Institute of Pathology, Washington, D. C. (Jones).

The Army Veterinary Research Laboratory was formerly located at Front Royal, Va.

*For management and dietary procedures, see the *American Journal of Veterinary Research*, 7, (Oct., 1946): 403-416.

cholesterol, inorganic phosphorus, calcium, potassium, chlorides, phosphatase, bilirubin, icterus index, total serum protein, serum albumin, serum globulin, fibrinogen, total red cell count, total white cell count, differential white cell count, and hemoglobin.

TABLE 2—Summary of Results of Serum Protein Values Found in Horses with Acute Iridocyclitis, Showing the Shift in the Albumin and Globulin Fractions



0=values for animals with normal eyes. Serum protein determinations were done on 15 normal horses. ●=values for animals with acute iridocyclitis. Fifteen serum protein determinations were done on 9 different horses.

METHODS

In general, the methods consisted of standard procedures commonly employed in routine laboratory analysis. In those cases in which the original techniques were designed for use with the visual colorimeter, they were modified in order to use the Fisher electrophotometer. In adapting visual colorimetric techniques to the electrophotometer, it was necessary to have the final volume of the test solution equal at least 25 cc. and to establish a standard calibration curve. From this curve, unknown sample readings were converted into quantities of the substance being determined. The reference curve for each determination was made by analyzing five or six graded dilutions of the standard. These were determined in triplicate. The average scale reading was plotted against its known concentration on graph paper and a line drawn between the points. The concentrations of unknown samples were found by referring to the graph. The electrophotometer was used for all determinations except blood proteins, in which case the visual colorimeter was chosen. In those determinations where protein-free blood filtrate was required, the method of Folin and Wu[2] was used for its preparation. The blood samples were drawn in the morning before the animals were fed and the filtrate prepared immediately.

Blood urea was determined by the method of Karr as modified by Northrup, Hawk, and Andes.[4] Total serum proteins, albumin, globulin, and fibrinogen were determined by the Andersch and Gibson[5] modification of the method of Wu and Ling, using the Klett visual colorimeter. Blood chlorides were measured by the volumetric method of Whitehorn.[6]

The acid hematin method was used for hemoglobin determinations. To establish a standard curve for the electrophotometer, a standardized blood sample was first prepared by determining the iron content of normal blood by the method of Wong.[7] With the iron content known, the hemoglobin in the sample was found by simple calculation. From this standardized blood of known hemoglobin concentration, a series of hematin solutions were made by diluting with N/10 hydrochloric acid. Electrophotometer readings of these solutions plotted against their hemoglobin concentration gave a standard reference curve. Analyses were performed by placing 0.02 cc. of whole blood in an electrophotometer cell containing 25 cc. of N/10 hydrochloric acid. After hemolysis was complete, a reading was taken and the hemoglobin concentration found from the calibration curve.

The method for determining the icterus index was similar to that used by Bernheim.[8] Clear serum was diluted 1 : 10 with physiologic solution of sodium chloride and then placed in the absorption cell and the electrophotometer reading taken. Conversion of the scale reading to units of icterus was done with the standard calibration curve described by Jones and Maurer.[9]

Nonprotein nitrogen and creatinine were determined by the methods of Folin and Wu[2]. Uric acid was determined by the method of Benedict[3]. In adapting this method to the electrophotometer, it was necessary to use 2 cc. of protein-free filtrate instead of 5 cc., and the final volume was made up to 25 cc. Potassium was determined in blood serum according to the method of Albanese and Wagner.[10] The only variation from their procedure was to increase the final dilution from 10 cc. to 25 cc. Cholesterol was determined by the methods of Bloor,[11, 12] modified for use with the electrophotometer. Phosphorus was determined by the method of Fiske and Subbarow.[13] Determinations were made on 3-cc. aliquots of filtrate resulting from the addition of 13.5 cc. of 10 per cent trichloracetic acid to 1.5 cc. of serum. The final volume was made up to 25 cc. The method of Roe and Kahn[14] was used for calcium. In this method, a trichloracetic acid filtrate was prepared exactly as for phosphorus. A 5-cc. aliquot was

used and the final volume was made up to 25 cc. Calcium was precipitated quantitatively as calcium phosphate and the phosphorus content determined. The standard curve for phosphorus was used to calculate calcium values. The amount of calcium per unit of serum is equivalent to the phosphorus per unit of serum times 1.16, minus the blank value. A blank using trichloracetic acid instead of blood serum was run through the entire calcium procedure. Bilirubin was determined by the Gibson and Goodrich[15] modification of van den Bergh's procedure. The amount of serum was changed to 1 cc., and the final volume made to 30 cc. Blood glucose was determined by the method of Folin and Wu.[16] A 1-cc. aliquot of protein-free blood filtrate was used. Bodansky's[5] method was used to determine phosphatase. Phosphatase values were obtained from the phosphorus standard curve. Each phosphatase unit represented 1 mg. of inorganic phosphorus converted from organic phosphorus by this enzyme.

The glucose tolerance test[17] was performed on horses with periodic ophthalmia and on horses with normal eyes. Each animal was fasted for eighteen hours and a blood sample taken for the fasting blood-sugar level. Then 600 Gm. of dextrose dissolved in 4 liters of water were given with a stomach tube. Venous blood was drawn thirty minutes, one hour, two, three, four, and five hours after glucose administration. The glucose content of each blood sample was determined by the method of Folin and Wu.[16] A curve was then plotted to show the blood glucose level in relation to time following administration.

Protein fractionations were done by the method of Howe.[18] No important changes were made in his technique.

The method of smearing and staining blood slides for differential leucocyte counts has been described by Maurer and Jones.[19] The combination Wright-Giemsa stain was used. Oxalated blood was drawn and smears made within thirty minutes. Total red and white cell counts were made within two hours after collection of blood.

CHEMICAL COMPOSITION OF THE BLOOD

Animals in the acute and quiescent stages of this disease were included in this study. These are recorded as having acute or quiescent iridocyclitis in table 1. In this table, column 1 is based upon 15 determinations on 9 animals with acute iridocyclitis. Six of these animals were tested in two separate acute attacks. Column 2 represents analyses on 15 animals in the quiescent stage. Column 3 is the average of one analysis on each of 15 horses with normal eyes.

With the exception of the serum proteins, there were no deviations found among the chemical blood constituents studied. Although physiologic variations occurred in the animals of each group, the average values for each group agreed closely. An exception was encountered: 1 animal in the group with acute iridocyclitis exhibited an icterus index and bilirubin value of a magnitude approaching clinical jaundice. These values were excluded from the tabulations since these changes were not attributed to ocular disease. It is of special interest to notice that no significant changes in the blood sugar or indications of marked change in glucose tolerance are evident in this table.

Serum Protein.—Serum albumin and globulin values differed in the acute iridocyclitis group in that the globulin value was increased at the expense of the albumin. This shift is further illustrated in table 2. The albumin-globulin ratio was often reversed although there was no change in the total serum protein.

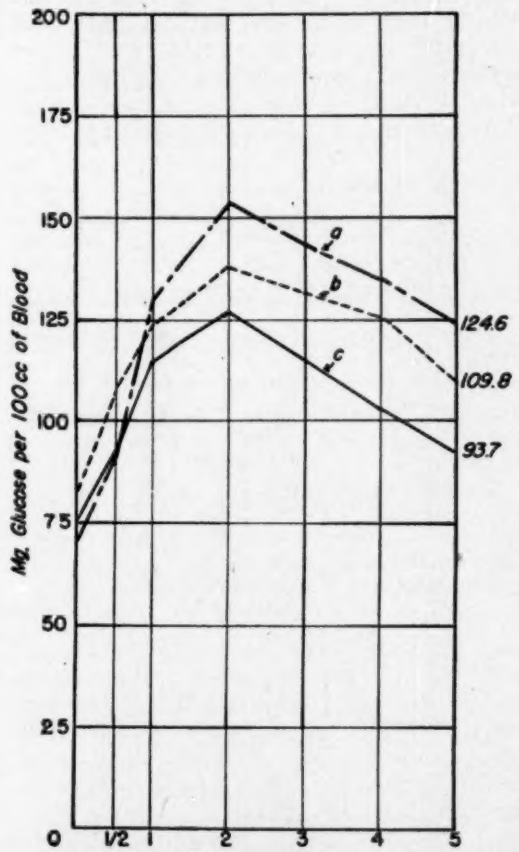
TABLE 3—Serum Protein Fractionation

No. Horses	Ocular Condition	Euglobulin Nitrogen	Pseudo-globulin I Nitrogen	Pseudo-globulin II Nitrogen	Total Globulin Nitrogen	Albumin Nitrogen	Non Protein Nitrogen	Total Nitrogen	Albumin-Globulin Ratio
10	normal	0.2213 ① (32%) ②	0.2456 (36%)	0.2289 (32%)	0.6958	0.6826	0.0342	1.4126	0.98:1.0
10	quiescent	0.3044 (36%)	0.3249 (38%)	0.2153 (26%)	0.8446	0.5619	0.0321	1.4386	0.67:1.0
7	acute	0.1720 (25%)	0.2721 (37%)	0.2759 (38%)	0.7200	0.5916	0.0324	1.3440	0.82:1.0

① All are average values expressed in grams nitrogen per 100 cc of serum
② Percentage figures in parentheses are approximate percentages of the globulin fractions

In normal animals, the albumin fraction generally exceeds the globulin fraction, and the resulting albumin-globulin ratio is from 1 : 1 to 2 : 1. In this group of 15 horses with normal eyes, only 2 had an albumin value less than the globulin. In these, the albumin was only slightly lower than the globulin fraction. Fourteen of the determinations done in the acute stage of iridocyclitis showed a higher globulin value than albumin. In the quiescent stage, this change was not always apparent in single analysis, although some difference can be seen in the average values. Repeated tests on the same animals later disclosed that alterations during the quiescent stage were also significant.

TABLE 4—Glucose Tolerance Tests in Horses with Iridocyclitis



a=average curve of tests in 10 animals with iridocyclitis, acute. b=average curve of tests in 10 animals with iridocyclitis, quiescent. c=average curve of tests in 10 animals with normal eyes.

In order to establish any possible correlation between the albumin-globulin ratio and the appearance of ocular symptoms, the serum proteins were studied further. Two horses which had been subject to severe attacks of iridocyclitis in both eyes were selected for this study. Determinations were made twice each week while the quiescent stage existed and on alternate days when the acute stage developed. The serum proteins were similarly studied in 2 normal animals. This experiment was continued until both horses with iridocyclitis went through a complete cycle of the disease; *i.e.*, from quiescent to acute and back to the quiescent stage. It was observed that both horses with periodic ophthalmia had consistently lowered serum albumin and proportionally elevated serum globulin; the albumin-globulin ratio in these 2 animals being about 0.75 : 1. As may be seen in chart 1, there was no constant correlation between the stage of ocular manifestations and this ratio. Chart 2 shows the blood proteins of a normal animal. The serum albumin generally exceeded the globulin but, occasionally, this ratio was reversed. However, the globulin never exceeded the albumin by a significant amount. The charts of the other 2 horses are similar and are eliminated for the sake of brevity. It is of interest to note that both normal horses had been maintained on a low riboflavin diet for several months, but neither had developed any ocular disease. The significance of the diet in this connection is unknown.

Protein Fractionation.—In order to determine which globulin fractions were increased, analyses were completed upon normal and affected horses. The results of this study are shown in table 3. Tests were made on the serum of 10 normal and 17 affected horses, 10 of the latter being in the quiescent stage, and 7 in the acute stage, of periodic ophthalmia. The average values are expressed in grams of nitrogen per 100 cc. of serum. In these animals, the lowered albumin-globulin ratio, previously discussed, is also evident in the affected groups. Considerable individual variation was encountered in this study, making interpretation of the average values somewhat difficult.

In the normal horses, the globulin fractions were nearly equal. In the quiescent

stage, the euglobulin and pseudoglobulin I fractions were increased in relation to the pseudoglobulin II. However, in the group of acute cases, the pseudoglobulin fractions were moderately increased at the expense of the euglobulin. The significance of these figures is unknown.

Glucose Tolerance.—Thirty glucose tolerance tests were completed. Ten horses with no eye disease were used, as were 10 horses in the quiescent stage. These were repeated on the affected animals as soon as acute symptoms reappeared. As may be seen in table 4, the oral administration of glucose was followed by hyperglycemia. The blood-sugar concentration then decreased as the liver and pancreas removed the glucose from the circulating blood. In animals with normal eyes, the peak of hyperglycemia occurred approxi-

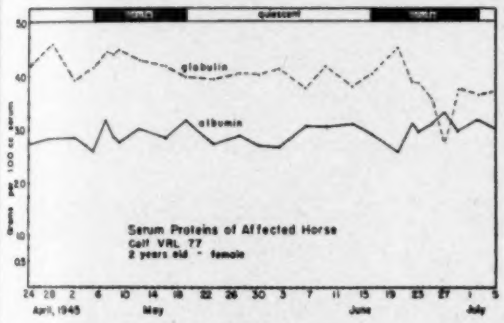


Chart 1

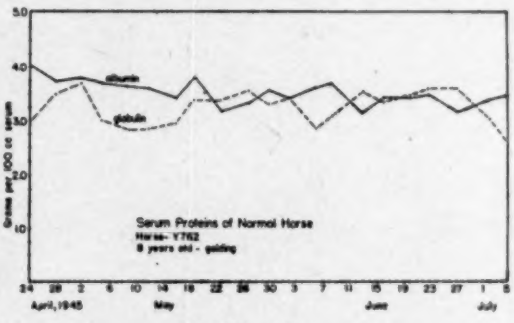


Chart 2

mately two hours after administration and declined gradually until, at the end of five hours, the blood was almost back to the original fasting level. In horses with iridocyclitis, blood glucose also attained its greatest concentration in two hours, but the height of the curve was much greater and hyperglycemia was of longer duration. At the end of the five-hour period, blood sugar was still well above the original fasting level. The glucose tolerance curves of horses with quiescent iridocyclitis were of the same pattern as in the acute stage, but they were not as pronounced in their divergence from the normal curve. From these results, it may be seen that horses with iridocyclitis have a decreased tolerance to glucose.

The significance of this finding is not readily apparent, although changes in glucose tolerance have been observed in some human diseases (tropical sprue, celiac disease). These are all considered of nutritional nature. Similar changes have been observed in hyperthyroidism. The changes seen in diabetes mellitus are of the same nature but much more severe.[21]

Cellular Elements of the Blood

Eleven horses with history of iridocyclitis were used in this study. During the quiescent period, counts were made twice a week but, when acute attacks developed, as was the case in 6 animals, counts were repeated each day until the ocular symptoms were again quiescent.

The most outstanding change found in the blood picture of horses with periodic ophthalmia was a relative lymphocytosis[22]. This increase in the number of circulating lymphocytes was at the expense of the neutrophils and usually most severe during the acute stage. No significant deviations from normal were observed in the other blood cells, nor in the hemoglobin. No change was seen in the Schilling classification of the neutrophils.

Charts of 3 cases selected from those studied will be presented to illustrate these findings.

Case 1.—Horse 219K, an 11-year-old gelding of riding type. This chart illustrates the leucocyte pattern of a horse with no history or lesions of ocular disease. It is readily apparent that, except for two occasions, the neutrophils were always greater in number than the lymphocytes. The total neutrophil count varied from 2,277 to 8,177 per cubic millimeter but was usually about 5,000 per cubic millimeter. The total number of lymphocytes varied between 1,680 and 4,002 per cubic millimeter. This appears to represent the normal blood picture of horses.

Case 2.—Horse Y18, a 5-year-old gelding of riding type. The blood pictures in all af-

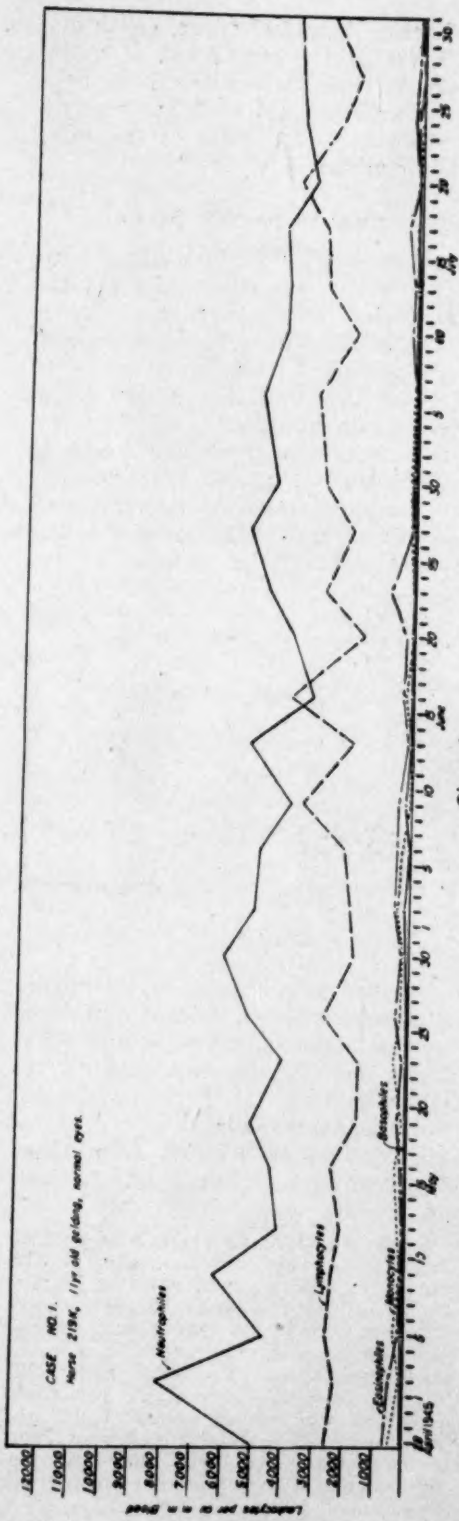


Chart 3

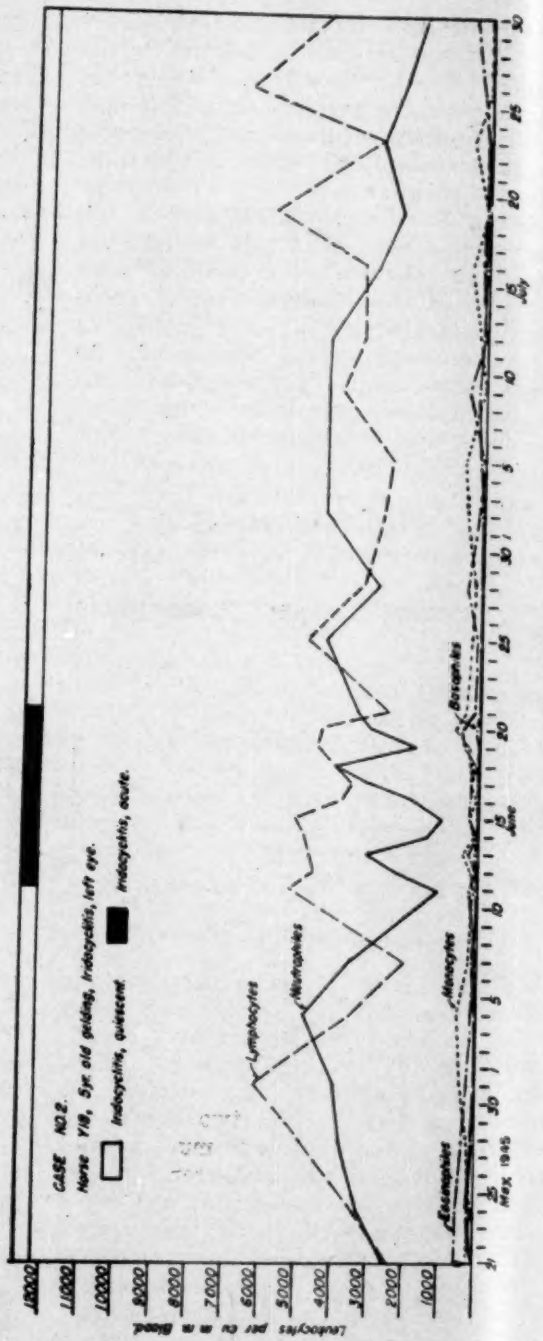


Chart 4

fected animals were similar to that of this horse. This animal had suffered four attacks of acute iridocyclitis in the left eye prior to the time these studies were started. The first of these attacks started on Jan. 25, 1944. On May 21, 1945, the lesions in the left eye

consisted of dark gray iris, posterior synechia, diffuse opacity of the lens, and many punctate and fibrillar opacities in the vitreous. It will be seen from the chart that an acute attack occurred during the course of these hematologic studies. Relative lymphocytosis was present during most of the time these observations were made. No other changes of significance were observed.

Case 3.—Horse VRL-77, 2-year-old filly of riding type. This animal developed periodic ophthalmia during her first year of life. When this blood study was started, there had been three or more acute attacks of iridocyclitis in each eye. Permanent lesions were present in both eyes. These consisted of diffuse corneal opacities, posterior synechiae, partial cataracts, gray discoloration of the iris, and many opacities in each vitreous. Blood counts were made regularly from April 28, 1945, to July 31, 1945, during which time there were three acute exacerbations. The right eye was involved in the first acute attack, starting on May 4. Acute symptoms appeared in the left eye on June 16 and again in the right eye on July 12.

Although a relative lymphocytosis was present through most of this study, severe increases in the number of lymphocytes were seen during the acute stage. This change was most pronounced during the last two acute attacks. The ocular symptoms in these attacks were unusually severe. The blood changes were correspondingly intense and illustrate those of a severe case.

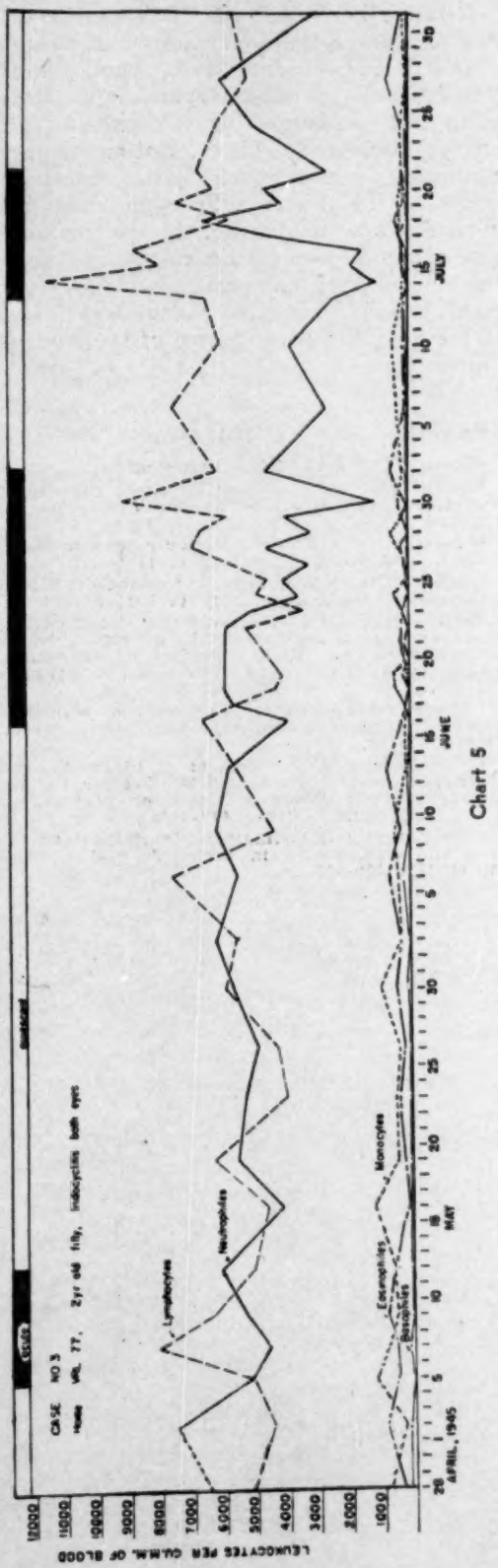


Chart 5

SUMMARY

Detailed studies were completed on the chemical composition and cellular elements of the blood of horses affected with periodic ophthalmia (recurrent iridocyclitis). Normal animals were also studied, using comparable techniques. Pronounced decrease in glucose tolerance was found in affected horses. Significant deviations were also found in the serum proteins. The globulins were usually increased at the expense of the albumin, the albumin-globulin ratio often being as low as 0.67 : 1.0 as compared with 1.0 or 2.0 : 1.0 in normal horses. Protein fractionation yielded variable results, with the euglobulin increased most in some and the pseudoglobulin in other cases. No significant deviations were observed in the blood content of nonprotein nitrogen, urea, creatinine, uric acid, glucose, chlorides, cholesterol, inorganic phosphorus, calcium, potassium, phosphatase, bilirubin, icterus index, total serum proteins, or fibrinogen.

Relative lymphocytosis, with concurrent neutropenia, was the outstanding change found in the blood picture. This change was found in all cases of periodic ophthalmia, but occasional counts would not always disclose it. This deviation was accentuated in some cases during the acute stage of the disease. No significant abnormalities were detected in the erythrocyte count or hemoglobin values, nor were the numbers of basophils, eosinophils, or monocytes affected. No change was found in the Schilling classification of the neutrophils.

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Immunologic and Clinical Studies on Plasma Fractions from Domestic Livestock. I. The Antibody Content of Porcine and Bovine Gamma Globulin

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THE INVESTIGATIONS reported herein and in subsequent papers are the logical sequels to the Harvard Medical School studies on fractionation of human plasma from blood collected by the American Red Cross. The characteristics of protein fractions of human plasma have been given by Cohn, Oncley, Strong, Hughes, and Armstrong.[1] That report was the first in a series describing chemical, clinical, and immunologic studies on the products of human plasma fractionation. In the series, Enders[2] reported on the concentration of antibodies in human globulin fractions for the following antigens: agglutinins against typhoid O antigen; agglutinins against H antigens; agglutinins against *Bacterium pertussis*, phase I; complement fixing antibody against influenza A virus, strain PR8; complement fixing antibody against the virus of mumps; inhibitor of the hemoagglutinin of influenza A virus; mouse protective (neutralizing) antibody against influenza A virus; mouse protective (neutralizing) antibody against swine influenza virus; mouse protective (neutralizing) antibody against virus of poliomyelitis; diphtheria antitoxin; streptococcal antitoxin; isoantibodies against cells of human blood groups A and B.

Not only Stokes, Maris, and Gellis[3] but Ordman, Jennings, and Janeway[4] discussed the clinical value of globulin in measles; both groups reported satisfactory results, with no complications arising from the injections.

Gellis, McGuiners, and Peters[5] studied the prevention of mumps orchitis by *gamma* globulin. The development of orchitis after the administration of the fraction derived from convalescent serum was compared with the results from normal serum. In the former, 7.8 per cent developed orchitis, compared with 27.4 per cent in the controls. With the fraction from normal serum, 20.9 per cent of the treated, and 28.8 per cent of the untreated, developed orchitis. The workers concluded that *gamma* globulin from normal plasma was ineffective in the dosage employed (50 cc.) in reducing the incidence, whereas the fraction from convalescent serum appeared highly effective.

TABLE 1—The Protective Value of Porcine Globulin Against Hog Cholera Virus

Pig	Inoculation. All received 2 cc. virus	Peak temp.	Result
1	10 cc. globulin	103.6	Lived
2	10 cc. globulin	104.0	Lived
3	4 cc. globulin	104.0	Lived
4	4 cc. globulin	103.6	Lived
5	2 cc. globulin	107.0	Died in 16 da.
6	2 cc. globulin	106.8	Lived
7	1 cc. globulin	107.0	Died in 11 da.
8	1 cc. globulin	106.8	Died in 13 da.
9	0 globulin	103.2	Died in 12 da.
10	0 globulin	105.2	Died in 16 da.

The vast supply of blood from domestic, food-producing animals available for fractionation probably contains neutralizing substances formed by exposure to various antigens. This paper, therefore, will report preliminary investigations on the antibody concentration in the fractions to certain animal pathogens.

EXPERIMENTAL

The *gamma* globulin used was in a sterile solution containing 16.5 per cent protein in 100 cc. of 0.3M glycine (amino-acetic acid) with no preservative. The composition of the protein, determined from electrophoretic pattern, was as follows: *gamma* globulin 97.5 per cent, and *beta* globulin 2.5 per cent. The blood from which the fractions were obtained represented a pooled sample from about 400 hogs or 400 cattle. The blood was collected

From the Department of Veterinary Science, University of California, Davis.

The fractions used in this study were made available by Armour and Company, Chicago.

without regard to postmortem or antemortem examination.

Hog Cholera.—Because, in this group of hogs, many would have been exposed to virus through vaccination, passive immunization against hog cholera suggested itself. Ten cholera-susceptible, crossbred pigs averaging 35 lb. apiece were divided into five lots of 2 pigs each. All were given 2 cc. of fresh hog-cholera virus. In addition, four lots received 10 cc., 4 cc., 2 cc., and 1 cc. of porcine *gamma* globulin, respectively. Lot 5, held as a control, received virus alone. Injections were made subcutaneously. Temperatures were taken daily, and autopsies were conducted on the pigs that died. The results are summarized in table 1, wherein are shown the peak temperature and the fate of each pig.

All pigs receiving 10 cc. and 4 cc. of *gamma* globulin survived and showed no significant rise in temperature. One of the 2 pigs receiving 2 cc. died of cholera; the other showed a significant rise in temperature, but lived. Pigs receiving 1 cc. of globulin were not protected and, with the controls, died of cholera.

Brucellosis.—Since the animals had probably been exposed to *Brucella abortus* or *Brucella suis*, a series of tube agglutination tests were conducted with Brucella as antigen, the fractions being in serial dilutions from 1 : 25 upwards. The results are summarized in table 2. As would be expected, no difference was observed between *Br. abortus* and *Br. suis* antigen. The bovine *gamma* globulin, however, agglutinated the antigen at a titer of 1 : 1,280, whereas, porcine *gamma* globulin agglutinated at 1 : 160. Porcine fraction I showed a titer of 1 : 5, compared with 1 : 80 for fraction III-1; but bovine fraction III-1 reacted at 1 : 160.

TABLE 2—The Brucella Agglutinating Titer of Normal Porcine and Bovine Plasma Fractions

Fraction	Antigen	Titer
Porcine γ-globulin P1C	*Br. abortus*	1 : 160
Porcine γ-globulin P1C	*Br. suis*	1 : 160
Porcine plasma P-1*	*Br. abortus*	1 : 5
Porcine plasma P-1*	*Br. suis*	1 : 5
Bovine γ-globulin C1823-A1	*Br. abortus*	1 : 1,280
Bovine fraction III-1 C180-A2	*Br. abortus*	1 : 160
Bovine plasma C-189*	*Br. abortus*	1 : 80

*Diluted 0.5 Gm. to 13.25 cc. H_2O.

The tests were duplicated with dried fractions from infected pigs. The results are shown in table 3, which also contains the composition of each fraction as determined from electrophoretic pattern. Fraction II contained 98.3 per cent *gamma* globulin; and when 1 Gm. was reconstituted to a volume of 26 cc., this fraction agglutinated *Br. abortus* at a titer of 1 : 3,200. Fraction III-1 with 98 per cent *beta* globulin showed a titer of 1 : 400. On a weight basis, 0.02 mg. of fraction II from infected pigs, as compared with 1.25 mg. from normal pigs, was required to agglutinate 1 cc. of antigen.

Staphylococcus.—The increasing importance of pathogenic staphylococci in animal health prompted an experiment to ascertain the antitoxin concentration in the fractions. Several strains of *Staphylococcus aureus* were titrated for toxin production by the method using hemolysis of rabbit erythrocytes as the index of toxicity. The organisms were grown on semisolid nutrient agar for forty-eight hours, after which the medium was thoroughly broken up and centrifuged. The supernatant fluid was decanted and, in varying dilutions, mixed with a 1.5 per cent suspension of washed rabbit erythrocytes. After the tubes had been incubated for one hour at 37.5 C. and refrigerated overnight, the degree of hemolysis was noted.

In titration of the antitoxin, the same procedure was followed, but with the addition of the plasma fractions to the tubes. Table 4 shows the results. Antitoxin was clearly demonstrated in the bovine *gamma* globulin,

TABLE 3—Agglutinating Titer and Composition of Plasma Fractions from Brucella Infected Swine

Fraction	Titer‡	Γ-Globulin	Fibrinogen	β-Globulin 2	β-Globulin 1	Γ-Globulin 2	Γ-Globulin 1	Albumin
Plasma	No test	25.4	9.8	13.6		17.7		33.5
I†	1 : 00							
II+III	No test	40.7	23.6	13.2		9.5	3.8	9.2
II+III W	No test	51.4	30.7	16.7		1.2		
II+III W Cent.	1 : 100	4.1		21.2		34.2		40.6
A Cent.	No test	68.8		12.2	14.9	2.8		1.3
III-2*	1 : 100	.....	83.4		7.3		1.7	9.6
III-1	1 : 400			73.6	24.4			2.0
II	1 : 3,200	98.3		1.7				
II+III Cent.	Neg.			13.8	13.3		6.0	66.9

*No separation of *gamma* and *beta* globulins; fibrinogen absent.

†Fraction insoluble; not analyzed.

‡One gram of fraction reconstituted to a volume of 26 cc. with distilled water. Composition from electrophoretic pattern.

to a lesser degree in the corresponding porcine fraction, and still less in the porcine I. It was absent in fraction III.

Since the strain of *Sta. aureus* used in this experiment was a stock culture of unknown origin, the fractions were titrated against other strains, including some of known bovine origin. In this experiment, the toxin was used at a constant dilution of 1 : 10, while the fraction dilutions varied from concentrated to 1 : 200. Three of the strains showed marked toxicity, two a slight amount, and four none at 1 : 10 dilution. The antitoxin concentration again was markedly higher in the *gamma* globulin than in the other fractions.

TABLE 4—The Antitoxin Action of Various Fractions Against a Staphylococcic Toxin as Titrated by Rabbit Erythrocyte Hemolysis

	0.2 cc. serum fractions + 0.4 cc., 1.5 per cent erythrocyte suspension								
	Bovine fraction				Porcine fraction				
0.2 cc. toxin	Γ-globulin		Γ-globulin		I		III		
dilution	conc.	1-100	conc.	1-100	conc.	1-100	conc.	1-100	Control
1 : 10	−	−	+	++++	++++	++++	++++	++++	++++
1 : 15	−	−	−	++++	+++	++++	++++	++++	++++
1 : 20	−	−	−	++++	−	++++	++++	++++	++++
1 : 25	−	−	−	++++	−	++++	++++	++++	++++
1 : 30	−	−	−	+++	−	++++	++++	++++	++++
1 : 40	−	−	−	+++	−	++	++++	++++	++++
1 : 50	−	−	−	++	−	++	++++	++++	++++
1 : 60	−	−	−	−	−	++	++++	++++	++++

conc.=concentrated fraction. ++++=complete hemolysis. −=no hemolysis.

DISCUSSION

When the neutralizing property of the porcine *gamma* globulin is bioassayed against hog-cholera virus, one obtains convincing evidence of the potential immunizing and therapeutic value of the *gamma* globulin fraction. This particular experiment was not conducted for the purpose of finding a substitute for hyperimmune serum; rather, the virus was chosen because hog cholera lends itself readily to passive immunization. The cost of producing the *gamma* globulin fraction alone would exceed that of hyperimmune serum unless uses were found for other fractions, such as albumin, fibrinogen, and fibrin foam. The last two have a use in surgery. Albumin has therapeutic value in conditions involving shock, loss of plasma, and dehydration. Heyl, Gibson, and Janeway,[4] studying the effect of concentrated solutions of human and bovine serum albumin on blood volume after acute blood loss in man, make the following statement: "There is ample evidence that concentrated human albumin is a safe and effective therapeutic agent. No statement regarding the safety for intravenous injection of crystallized bovine serum albumin of low globulin content can be made until more extensive clinical tests have been completed in order to determine to what extent and under what circumstances it will be safe to use a protein of animal origin for therapy in man."

Since the antibodies are concentrated in the globulin fractions, which are highly antigenic, they should be used on the homologous species. On the other hand, the albumin fraction, not used for its antibody content, and being low in antigenicity, could probably be used with safety on another species.

The Brucella agglutinating property of the porcine fraction is interesting. A group of pigs, such as those from which plasma was obtained, would show not more than 1 per cent reactors at titer of 1 : 25 in the agglutination test on blood serum. The relatively higher incidence of brucellosis in cattle than in swine, and the resultant heavier exposure, are confirmed in the higher titer found in the bovine fraction. In addition, the pig is less efficient in agglutinin production than the cow. Passive immunization has not been successfully used in brucellosis of cattle or swine, and whether or not the concentrated antibody can be effective will be

determined by animal experimentation. Apparently, however, Brucella agglutinins are concentrated in the *gamma* globulin.

In all probability, the greatest application of the globulin fractions will be found in those classes of livestock, such as chickens, turkeys, and rabbits, where extensive domestication is relatively recent, where large numbers are concentrated on small areas, and where mortality from infectious disease constitutes the greatest single hindrance to economical production. An application may also lie in the field of pork production, wherein it has been stated that 40 per cent of pigs farrowed fail to reach market. Though the etiology of some losses is known, that of others is obscure and cannot be titrated *in vitro* with the globulin fraction. In such instances, the effectiveness must be tested clinically. The use of plasma fractions in therapy, and passive immunization leading to modification, at least, of an outbreak of disease, would therefore appear as a distinct possibility. Intensive investigations exploring this field are warranted.

SUMMARY

1) A bioassay on the neutralizing value of the *gamma* globulin fraction derived from plasma from abattoir pigs showed that 35-lb. pigs were protected from 2 cc. of virus by 4 cc. of the fraction.

2) The same material agglutinated *Brucella abortus* and *Brucella suis* at a titer of 1 : 160. The fraction from bovine plasma gave a titer of 1 : 1,280. Tests with other fractions showed conclusively that the agglutinins for Brucella are contained in the *gamma* globulin.

3) Staphylococcic antitoxin was demonstrated in the bovine *gamma* globulin and, to a lesser degree, in the porcine. It was absent in other fractions.

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Concentrations of Penicillin in the Bovine Mammary Gland Following Infusion and the Penicillin Tolerance of Certain Streptococci

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THE DEVELOPMENT of penicillin prompted extensive trials of its use for the treatment of acute and chronic bovine mastitis. Many reports on the wide range of dosage and dilution, and the clinical and bacteriologic results obtained, have been published. Little appears to have been published, however, on the concentration of penicillin obtained and its persistence in the mammary gland as influenced by unit dosage and volume of the material injected.

Weirether, Jasper, and Petersen[1] carried on some experiments with a small number of quarters to determine its toxicity and subsequent behavior after infusion into the mammary gland. Although the number of cows used was small, indications were that an aqueous solution of penicillin sodium did not have any irritating properties when so infused. In view of this situation, it was thought that a detailed study of a wide range of dosages, each

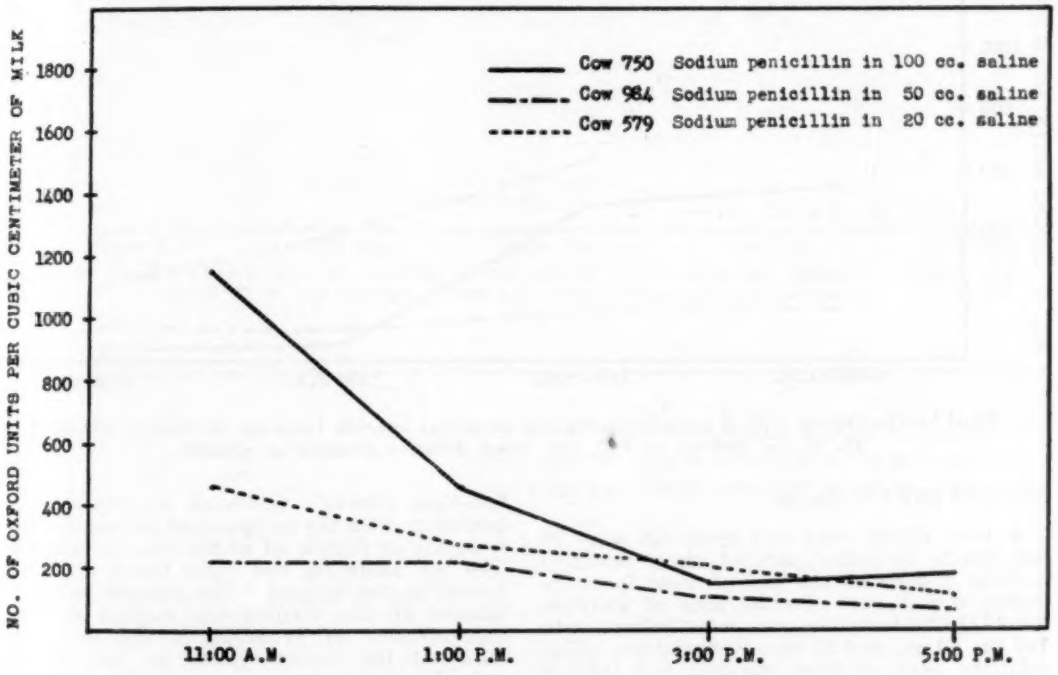

Chart I—The Oxford units of penicillin in the milk at various intervals following injection of 200,000 O. U. per quarter at 9:00 a.m. using different amounts of diluent.

Authorized for publication on Aug. 15, 1946, as Paper No. 1337 in the Journal Series of the Pennsylvania Agricultural Experiment Station from the Animal Pathology Laboratory.

Presented before the Section on Research, Eighty-third Annual Meeting, American Veterinary Medical Association, Boston, Mass., Aug. 18-22, 1946.

From the Department of Animal Husbandry (Thorp and Straley); and the Department of Bacteriology (Uhrik), Pennsylvania State College, State College.

given with varying amounts of diluent, should be made. The investigation herein reported is primarily concerned with the maintenance of an adequate concentration in the milk following infusion.

ment of Bacteriology of this station made the leucocyte determinations and carried out the *in vitro* tests on the effect of penicillin on certain strains of streptococci.

Milk samples for assay were taken from each quarter of all the experimental cows at

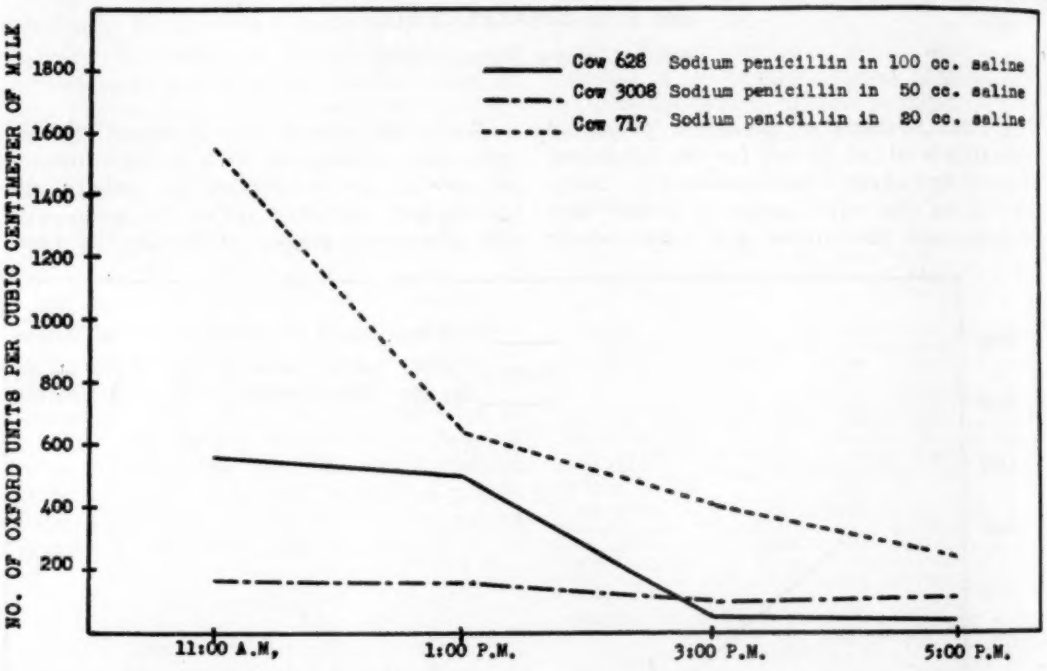

Chart 2—The Oxford units of penicillin in the milk at various intervals following injection of 100,000 O. U. per quarter at 9:00 a.m. using different amounts of diluent.

METHODS AND PROCEDURE

A total of 45 cows was used; 36 were in the middle to latter part of their lactation periods, 5 were at the end of their lactation period, and 4 were chronic cases of mastitis. Six of the 36 lactating cows were infused with 100 cc., 50 cc., and 20 cc. of physiologic saline solution; each of these amounts was infused into all quarters of 2 animals to determine the effect of various amounts of diluent alone on milk production and the number of leucocytes in the milk.

Throughout this manuscript, Oxford units will be referred to as "units," and the physiologic saline prepared with water double-distilled over glass will be the "diluent." In the first tests, 6 cows were used. All of their quarters were infused with 200,000 units of penicillin, 2 receiving it in 100 cc., 2 in 50 cc., and 2 in 20 cc. of the diluent. This procedure was repeated in four other series of 6 cows each, using 100,000, 50,000, 25,000, and 10,000 units per quarter and the same amount of diluent.

Milk samples were collected for microscopic examination just before, and forty-eight hours and one week following, infusion. The Department of Bacteriology of this station made the leucocyte determinations and carried out the *in vitro* tests on the effect of penicillin on certain strains of streptococci.

Milk samples for assay were taken from each quarter of all the experimental cows at two-hour intervals following the injection of penicillin, with the exception of the cows which were dry or drying off at the time of infusion. The last sampling was eight hours after the penicillin was infused. The samples were examined by the Oxford cup method for the concentration of penicillin in the milk. In many of the recent reports on its use for animals, there was some variation of the methods used to determine penicillin in the milk or blood. That, in our opinion, justifies a detailed account of the methods used for this investigation.

The material to be tested for the amount of penicillin present was diluted with a phosphate buffer, pH 6.5, to obtain a final concentration approximating 1 unit per cubic centimeter. If the potency was unknown, several dilutions were made.

Plates were prepared by pouring into sterile Petri dishes 22 cc. of sterile nutrient agar, which was allowed to harden. Nutrient agar, in small flasks, was melted, held at 48 C. in a water bath, and seeded with 1 cc. of the test organism culture of *Staphylococcus aureus* per 100 cc. The cultures had been grown in a nutrient broth at 37 C. and were from

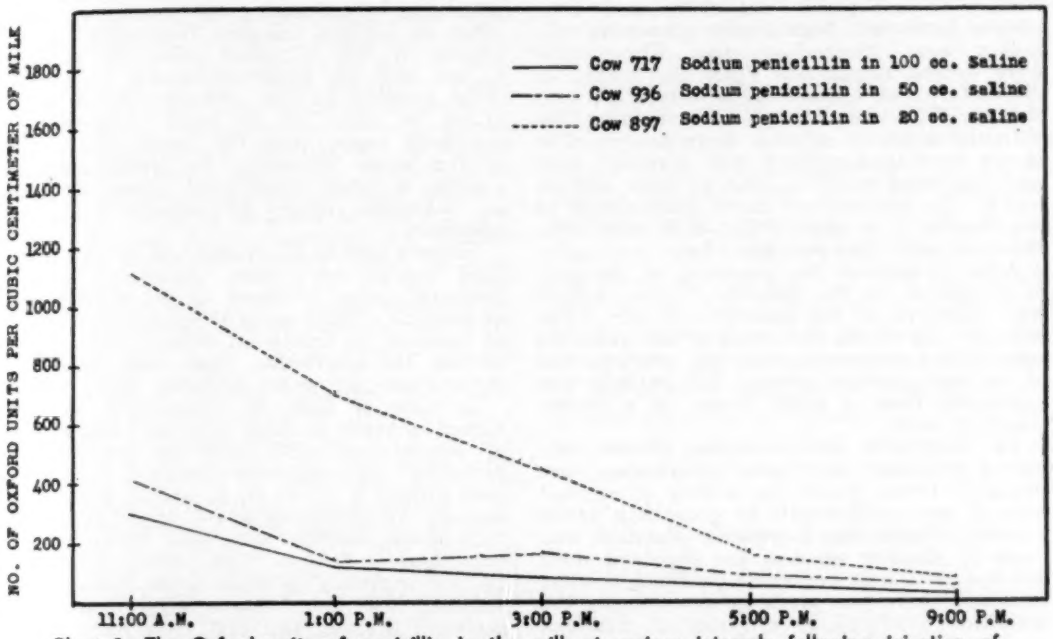

Chart 3—The Oxford units of penicillin in the milk at various intervals following injection of 50,000 O. U. per quarter at 9:00 a.m. using different amounts of diluent.

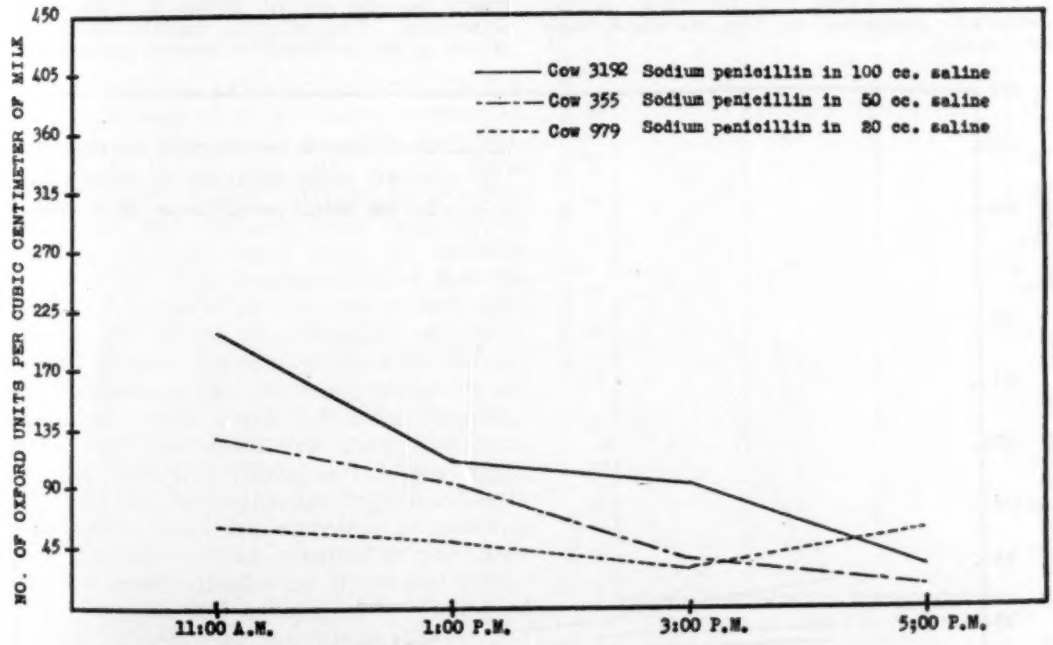

Chart 4—The Oxford units of penicillin in the milk at various intervals following injection of 25,000 O. U. per quarter at 9:00 a.m. using different amounts of diluent.

twenty-four to thirty hours old. Three cc. of the seeded agar was spread evenly over the surface of each plate. Before the agar completely hardened, four sterile aluminum cylinders were dropped on top. These cups were spaced equidistant from the center of the plate. Two opposite cups were filled with the working standard and the remaining two with the unknown solution to be tested. The plates were then covered with porcelain tops and incubated at 37 C. for at least sixteen hours. To prevent too rapid evaporation in the incubator, a glass Petri dish cover was inverted over the porcelain top.

After incubation, the diameter of the zone of inhibition of the growth of *Sta. aureus* was measured to the nearest 0.25 mm. The average size of the two zones of the unknown sample was compared with the average size of the two standard zones. The reading was calculated from a chart based on a known standard curve.

The standard stock solution, which contained 50 units per cubic centimeter, was prepared twice weekly by adding a weighed amount of penicillin salt to phosphate buffer solution. Each day, a working standard was made by diluting some of the standard stock solution to 1 unit per cubic centimeter. The standard curve was made by determining the inhibition produced by a series of dilutions of the standard solution. The zone sizes were determined for 0.25-, 0.5-, 1.0-, and 2.0-unit solutions and the curve plotted. The average size of the standard zones on each plate was plotted on the curve and the value of the unknown, compared to the standard, was determined.

On milk samples, the cup assay seemed to give acceptable results. As there was some difficulty in estimating the amount of penicillin in various samples, frequently the dilutions of the unknowns were not so close to one unit per cubic centimeter as desired. This resulted in the unknown zones being either so large or so small, compared to standard zones, that the assay figure was only a rough estimate. To assure accurate results, a large number of plates covering an adequate range of concentration was necessary.

Various strains of streptococci of the Lancefield groups have been considered as the bacterial agent involved in the development of mastitis. Some cases of mastitis are known to respond to treatment better than others, leaving the impression that some strains of streptococci are more resistant than others.

In view of this, an experiment was designed to make *in vitro* tests on some strains of streptococci with different amounts of penicillin; representative strains of the Lancefield groups A, B, C, D, E, and G, plus intermediate *Streptococcus uberis* types associated with bovine mastitis, were used. These streptococci were as follows: C203, group A, a stock culture originally obtained from Temple University; N49 from Storrs Agricultural Experiment Station, and 27R from an isolation made by the bacteriology laboratory of this station, both Group B; *Streptococcus dysgalactiae*, group C, from C. S. Bryan of Michigan State College; Cl, D76, group D; K129, group E; and D166B, group G from R. C. Lancefield. Two cultures, namely B1747 and B1400 of *Str. uberis* from Storrs Agricultural

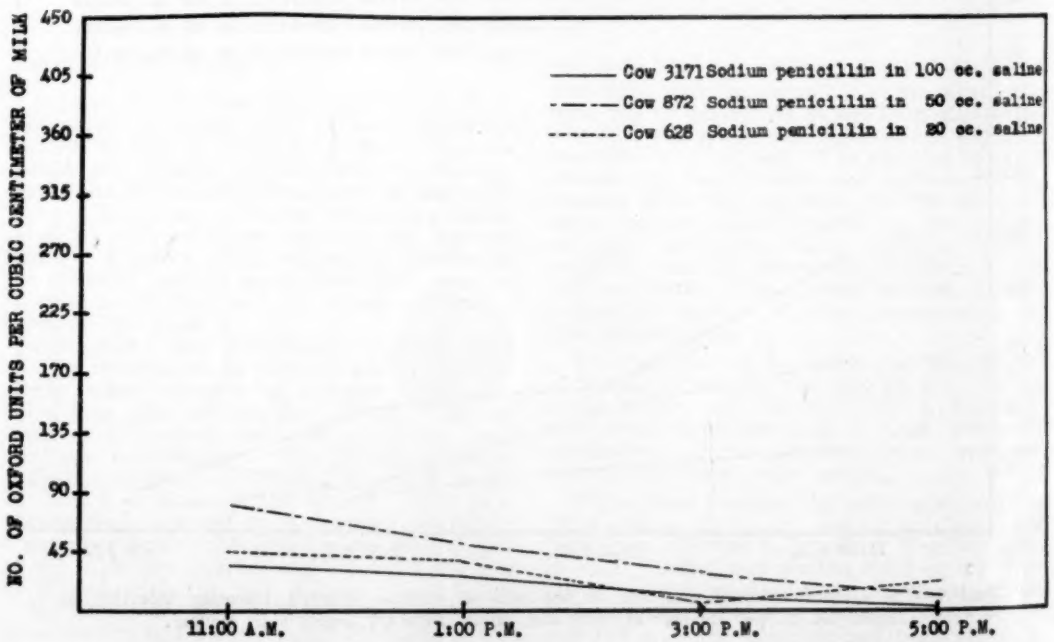

Chart 5—The Oxford units of penicillin in the milk at various intervals following injection of 10,000 O. U. per quarter at 9:00 a.m. using different amounts of diluent.

Experiment Station, were classified as group D by the bacteriology laboratory of this station.

Dilutions for the *in vitro* tests were made by dissolving 200,000 units of penicillin sodium in 500 cc. of sterile saline to give a final concentration of 400 units per cubic centimeter. From the stock solution, dilutions of the following concentrations were made in veal infusion broth: 200, 100, 50, 10, 5, and 1 unit per cubic centimeter. Those strains which were unable to tolerate 1 unit per cubic centimeter required dilutions of 0.5, 0.1, and 0.05 units per cubic centimeter for further tests. These mediums were tubed in 5-cc. amounts in tubes 0.5 in. by 5.0 in. After preliminary trials, it was found that 0.5 cc. of an eighteen-hour broth culture gave more consistent results than using a loopful of the eighteen-hour culture.

The tubes containing penicillin broth were inoculated immediately after dilutions were made and incubated at 37 C., being checked for growth at twenty-four and forty-eight hours. At the end of forty-eight hours, 1.0 cc. of each culture was transferred to 5-cc. veal infusion broth tubes, containing no penicillin and incubated at 37 C., and observed for growth at twenty-four and forty-eight hours. Both sets of cultures were observed daily for growth for one week before recording final results. Inactivation of penicillin was tried on the first set of cultures tested, according to the methods of Muir and Valley,[3] but no advantage was found nor were better results obtained.

DISCUSSION

The infusion of penicillin into the bovine mammary gland presents a number of variables which should be taken into consideration in the evaluation of this data. To eliminate as many of these as possible, the cows were infused with penicillin in groups of 3 as nearly equal in lactation as possible. The results indicated that the stage of lactation or quantity of milk produced, the extent of induration and, to a lesser degree, the size of the milk cistern, all influenced the units of penicillin remaining eight hours following injection. Weirether and associates[1] state that some of the reduction following infusion might possibly be due to pyrogenic properties in the water used for diluent. An attempt to eliminate such an eventuality was made by the double distillation of the water over glass and careful handling and rinsing of the apparatus used for injection .

The results obtained from the periodic examination of the milk for penicillin following infusion of the various dosages into the mammary gland of lactating cows are shown in charts 1 to 5, inclusive. These values represent the average concentration for all quarters, although individual quarter samples were assayed.

The data shown in charts 6 and 7 are the results of infusing penicillin at two widely different dosage levels into 5 dry cows ending their lactation period. In the case of cows 820, 930, and 3134 (chart 6), 200,000 units were infused; of the 12 quarters, there was a significant concentration of penicillin left in nine at seventy-two hours. In the cows infused with 50,000 units of penicillin under the same conditions, there was a decided difference in the units remaining at forty-eight and seventy-two hours (chart 7). This procedure of injecting the mammary gland at the end of the lactation period may have

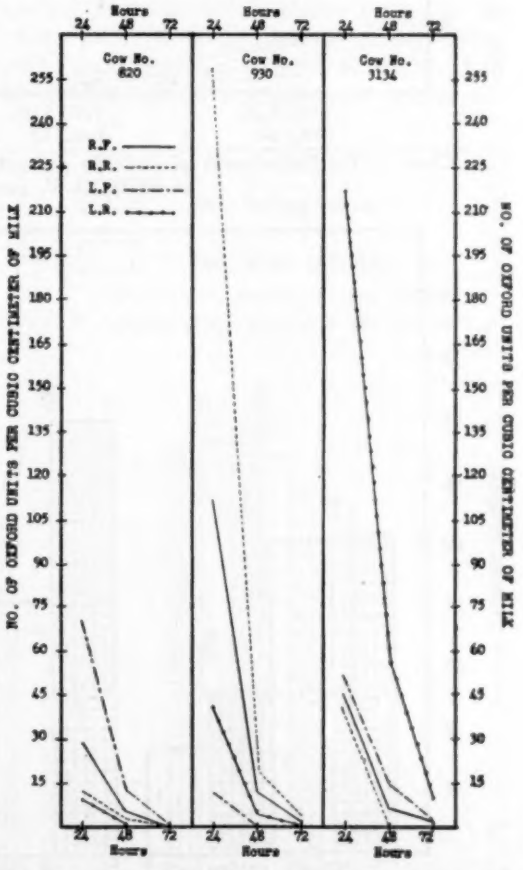

Chart 6—The Oxford units of penicillin in the milk at 24-hour intervals following injection of 200,000 O. U. per quarter at last milking ending the lactation period.

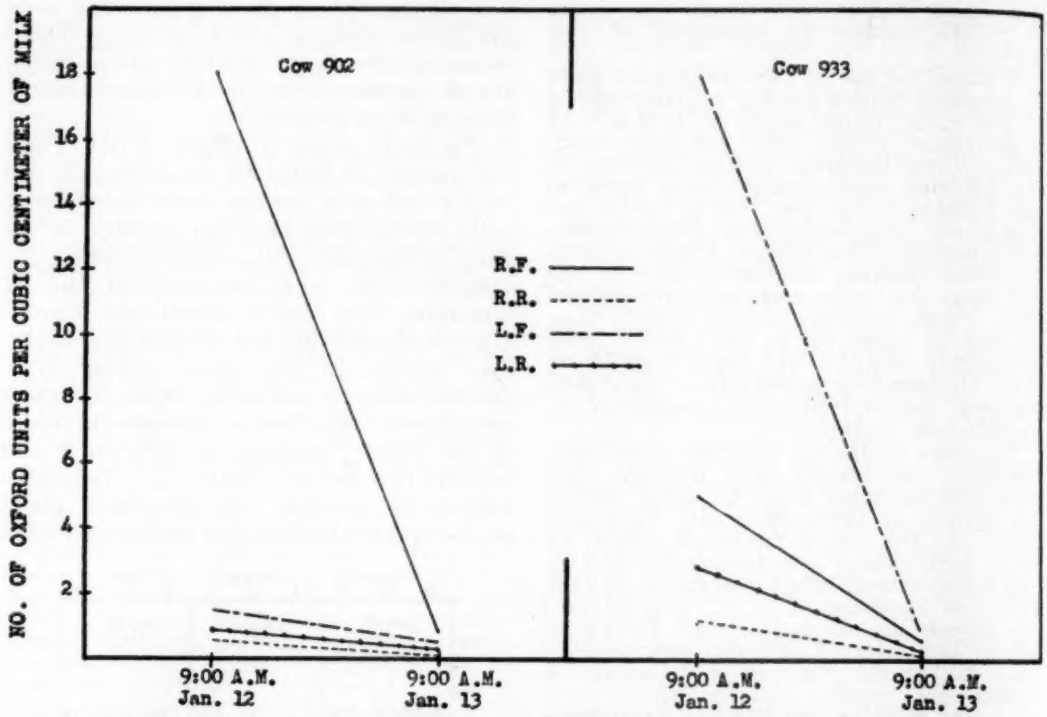

Chart 7—The Oxford units of penicillin per quarter at 24- and 48-hour intervals following injection of 50,000 O. U. per quarter in 2 dry cows.

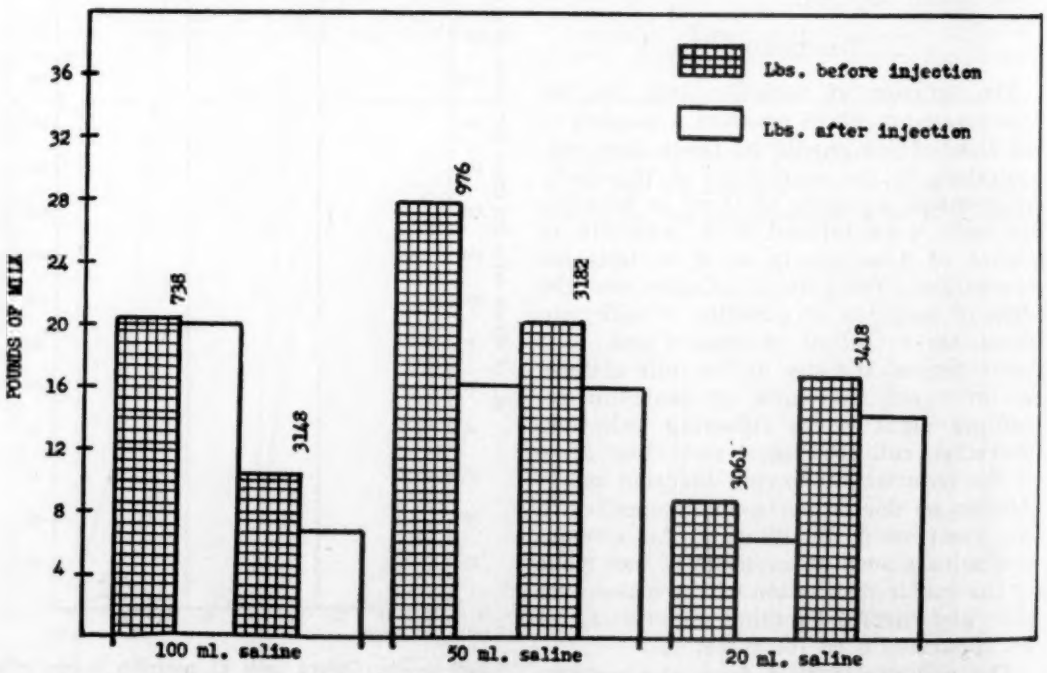

Chart 8—The average pounds of milk produced for three days prior to injection compared to the three-day average following the injection of various amounts of physiologic saline in each quarter.

definite merit in those cases which do not respond to other methods of penicillin treatment.

As the infusion of physiologic saline alone had an effect upon milk production, probably some of this change was due to the increased intraglandular pressure. It appears from a study of the data (charts 8 and 9) that there is no marked difference in the effect on milk production between the infusion of the same volume of physiologic saline solution.

Examination of milk smears before and after infusion was made to determine the effect on the number of leucocytes in the milk forty-eight hours and one week after. No difference was observed between the cows receiving the same amounts of physiologic saline and the two higher dosages of penicillin.

The group D enterococci, C-1, D76, and B1747 (Storrs' *Str. uberis* type), were found to tolerate at least 200 units of penicillin per cubic centimeter; higher concentrations were not tested. The *Str. agalactiae*, N49 and 27R, and the *Str. dysgalactiae* strain were not able to tolerate more than 1 unit of penicillin. Strains K129 (group E) and D166B (group G) tolerated 0.5 and 1.0 unit per cubic centimeter, respectively. The strain of Lancefield group A was able to grow when transferred from a 10-unit per cubic centimeter concentration of penicillin, although visible growth in the actual presence of penicillin was noted in a concentration of only 1 unit per cubic centimeter. Strain B1400, a *Str. uberis* type, was able to grow in the presence of 1 unit per cubic centimeter. At one time, a tolerance of 10 units per cubic centimeter was developed; higher concentrations were not tried. These results (table 1) would indicate considerable variation in the activity of penicillin against some of the bacteria found in the udder and, in many instances, associated with mastitis. On the other hand, bacteria such as *Str. agalactiae*, which are

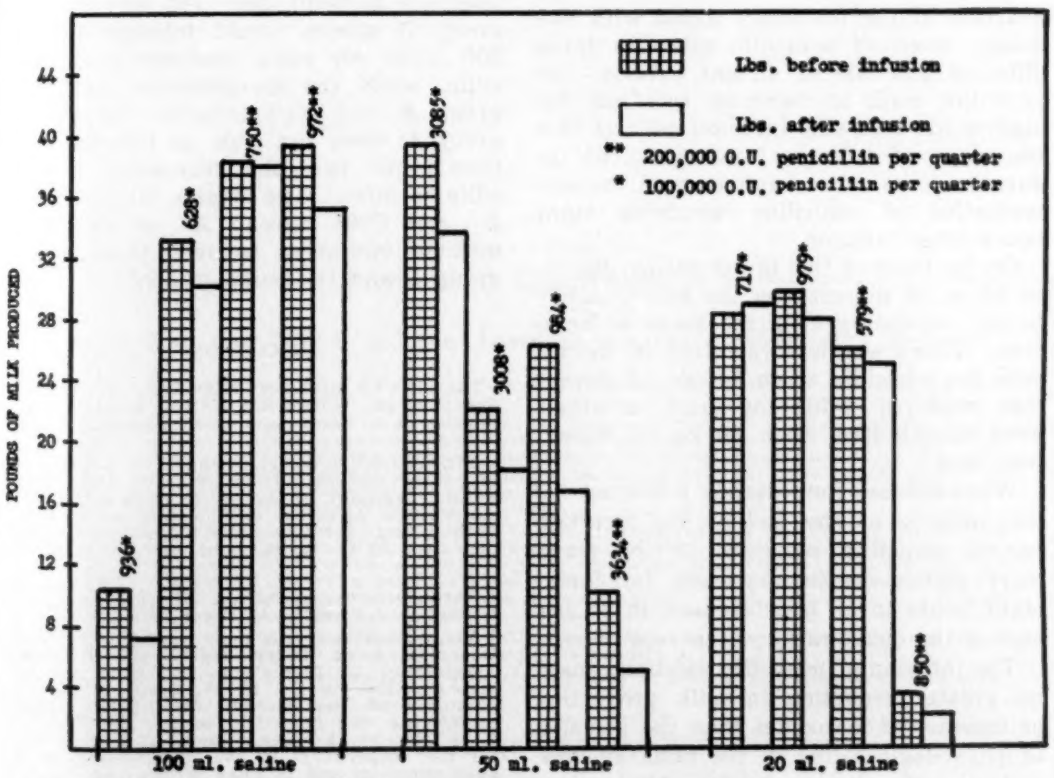

Chart 9—The effect of udder infusion with penicillin and saline at various dosages upon the average milk production.

considered by many as one of the etiologic agents of mastitis, will not tolerate high concentrations of penicillin and are comparatively sensitive to this antibiotic.

cate sufficient increase in concentration of penicillin at the end of eight hours over those receiving 100,000 units to justify the higher dosage.

TABLE I—The In Vitro Effect of Penicillin on Selected Strains of Streptococci

Strain	Growth in presence of penicillin			Inhibited but not killed by penicillin in 48 hr.
	24 hr. units/cc.	48 hr. units/cc.	1 week units/cc.	units/cc.
C-1 (group D)	10	100	200 *	200 *
D76 (group D)	10	100	200 *	200 *
B1747 (group D)	10	100	200 *	200 *
B1400 (group D)	1	1	1	10 *‡
N49 (group B)	..	0.5	1	1—5
27R (group B)	..	0.5	1	1—5
K129 (group E)	..	0.1	0.5	0.5—1
Str. dysgalactiae (group C)	..	0.1	0.5	1—5
C203 (group A)	..	0.1	1	10—25
D166B (group G)	..	1	†	1—5

*Higher concentrations of penicillin were not used.
†Consistent results were not obtained in this test.
‡A tolerance was developed over a period of three weeks.

SUMMARY

Forty-five cows were infused in all quarters of the mammary gland with five dosage levels of penicillin given in three different amounts of diluent. Assays for penicillin made at two-hour intervals for eight hours following infusion indicate that the stage of lactation and extent of induration have a direct influence on the concentration of penicillin remaining eight hours after infusion.

On the basis of this investigation, 100 cc. or 50 cc. of diluent was the best quantity to use, depending upon the stage of lactation. There was less variation in the assays for penicillin when 100 cc. of diluent was employed, while the most variations were encountered when 20 cc. of diluent was used.

When infused immediately following the last milking at the end of the lactation period, penicillin remained in the mammary glands of the dry cows for forty-eight hours in all quarters and in 75 per cent of the quarters for seventy-two hours.

The infusion of penicillin solution caused no greater reduction in milk production or increase of leucocytes than the infusion of physiologic saline in the same amount.

Assays of milk samples from those quarters receiving 200,000 units did not indicate sufficient increase in concentration of penicillin at the end of eight hours over those receiving 100,000 units to justify the higher dosage.

The strains of the Lancefield groups studied *in vitro* indicated two extremes of tolerance to penicillin. The enterococci group D strains would tolerate at least 200 units per cubic centimeter of penicillin, while the *Streptococcus agalactiae* group B and *Streptococcus dysgalactiae* group C were not able to tolerate more than 1 unit per cubic centimeter of penicillin *in vitro*. The strains B1400 (group D) and C203 (group A) showed intermediate tolerances between those of the group B and D strains studied.

DISCUSSION*

DR. H. L. BROWN (Pawling, N. Y.).—Dr. Thorp, I am wondering if you have had any experience in using penicillin in small amounts, such as 10 cc.?

DR. THORP.—We ran assays on a few cows in our preliminary work. I think that in the smaller amount there is a chance for this material to lie in the milk cistern of the mammary gland. At least, the assays would indicate that, in the animals in which a high pro-

*After the presentation of the paper, the following discussion was conducted.

The authors express their appreciation to the Department of Dairy Husbandry for their coöperation in furnishing the cows for this investigation; to Dr. R. W. Stone of the Department of Bacteriology for his coöperation in making the penicillin assays; to Dr. J. J. Reid, also of the Department of Bacteriology, for his coöperation in the examination of the milk samples; and to Chas. Pfizer and Co., Inc., Brooklyn, N. Y., for furnishing the penicillin and research grant in partial support of these studies.

duction is not found. In other words, the milk is another diluent. The penicillin, I think, will diffuse readily through the milk, and it is stable in the milk.

CHAIRMAN STUBBS.—Someone made a statement earlier in the meeting that little work was being done on dosage. Surely some of you will want to question Dr. Thorp on that matter.

DR. J. W. WALKER (Holmeville, Pa.).—I would like to know if any bacteriologic tests were made in these cases, if they responded, and if there was any difference in the response.

DR. THORP.—Of the 45 cows used, there were only 8 or 9 on which we had bacteriologic reports. We are going to report the clinical phases of it in a separate paper. With the higher dosage, we had a little better response than with the lower dosage; more so where one dose was given than where the intermittent dose of 50,000 or 25,000 units was given.

We made bacteriologic tests on those cows that were treated in our own herd.

DR. RONALD GWATKIN (Hull, Que.).—In the work done in the Division of Animal Pathology, I think we are pretty much in agreement that the 100-cc. quantities gave a larger percentage of recoveries.

DR. WALKER.—What diluent did you use?

DR. GWATKIN.—Saline.

References

[1]Weirether, F. J., Jasper, S. E., and Petersen, W. E.: Effect of Infused Penicillin in the Bovine Mammary Gland. Proc. Soc. Exptl. Biol. and Med., *59*, (1945): 282.

[2]Muir, R. D., and Valley, G.: A Suggested Sterility Test for Penicillin. Science, *101*, (1945): 390.

Notice to Contributors

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Manuscripts.—Manuscripts must be typewritten, double-spaced, and the original, not the carbon copy, submitted. One-inch margins should be allowed on the sides, with 2 in. at top and bottom. Articles should be concise and to the point. Short, simple sentences are clearer and more forceful than long, complex ones. Footnotes and bibliographies **also** should be typed **double space** and should be prepared in the following style: name of author, title of article, name of periodical with volume, month (day of month, if weekly), and year.

Illustrations.—Photographs should be furnished in glossy prints, and of a size that will fit into the Journals with a minimum of reduction. Photomicrographs which cannot be reduced should be marked for cropping to 1-column or 2-column width. Drawings should be made clearly and accurately in India ink on white paper. Figures appearing on graphs or charts should be large enough to allow for reduction necessary for the chart or graph to fit on Journal pages. All illustrations should bear the name of the author on the back.

Tables.—Tables should be simple. Complex tables are not conducive to perusal. It is wiser to summarize complex material rather than to attempt to tabulate it.

News.—Secretaries of associations and readers are requested to send us announcements of meetings and news items.

AMERICAN VETERINARY MEDICAL ASSOCIATION

600 So. Michigan Avenue, Chicago 5, Illinois

Permanent Enteric Fistulas for Studies of Ruminant Digestion, Assimilation, and Elimination

R. E. NICHOLS, D.V.M., Ph.D.

Lafayette, Indiana

IT IS POSSIBLE, by producing permanent fistulas at various levels of the enteric tract, to establish a group of animals from which samples of ingesta may be drawn at frequent intervals from many different portions of the tract while the group is being fed any ration of choice. As an adjunct to the chemical analyses of feeds and excretions, this method of making "laboratories" of the animals themselves offers the possibility of more accurate study of the physiology of normal digestive and assimilative processes. Pharmacodynamic phenomena for substances other than feed can also be investigated.

Fig. 1—Rumen fistula covered by plug.

Fig. 2—Small intestine fistula.

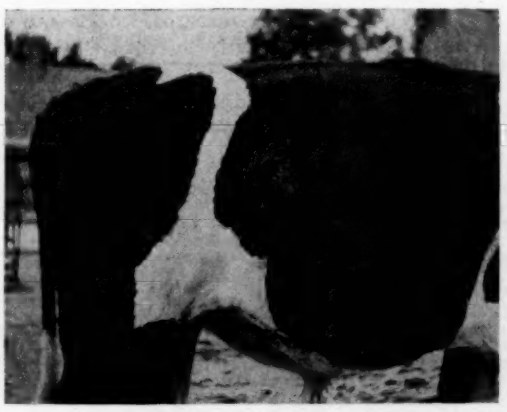

Fig. 3—Cecal fistula.

Fig. 4—Colon fistula.

Published as Journal Series Paper No. 265 of the Purdue University Agricultural Experiment Station.

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Cattle lend themselves readily to this kind of surgery. The technique for establishing a rumen fistula (fig. 1) has been adequately described by Schalk and Ama-

don.[1] For a fistula of the small intestine (fig. 2), one can employ enough chloral hydrate to make the animal unsteady on its feet and, after it has been thrown, enough nembutal intravenously for complete relaxation. While the animal is down, considerable positive pressure in the abdominal cavity can be avoided by placing a large hypodermic needle through the wall of the abdomen and of the rumen at the topmost position when the animal is on its side. If left in place during the operation, this allows the gas to escape. The fistulas of the cecum (fig. 3) and colon (fig. 4) are accomplished under nembutal sedation and local procaine infiltration. The fistulas in the intestines are lateral, that is, the mucous edges of an opening in the side of the bowel are fastened to the skin edges of the incision with, of course, sufficient surrounding supporting sutures. The intestinal fistulas are made small enough so that when they are healed the ingesta in the opening will tend to dry up between sampling times and, at least partially, plug the openings, thus minimizing the large loss of nutrition which accompanies larger openings or other types of fistulas where ingesta which the animal needs for maintenance leaks out. With good technique, post-infection dangers are practically *nil*, but care is necessary to prevent too much drying and chilling of mucous surfaces, particularly in cold weather.

[1]Schalk, A. F. and Amadon, R. S.: Physiology of the Ruminant Stomach. North Dakota Agric. Exper. Sta. Bull., 216, 1928.

Milk Fever (Parturient Paresis) as a Manifestation of Alkalosis

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Towanda and Rome, Pennsylvania

EVIDENCE has been presented to support the concept that the determining cause of milk fever (parturient paresis) is alkalosis.[1, 2] Futhermore, it appears likely that ketosis, or acetonemia, which occurs either as a complication of milk fever or as a separate disease, may be regarded as another manifestation of alkalosis. The present study was planned to test this concept against cases of milk fever as they occurred in practice. This report reviews a series of cases treated with Chlor-Ethamine, an acidifier for oral administration, supplementary to intravenous calcium therapy.

A relationship between milk fever and acetonemia may be suspected from the circumstances of their occurrence. While these diseases occur independently, it is not uncommon for them to be associated, or for acetonemia to follow recovery from milk fever. Routine examination of the urine in milk fever reveals many cases with acetonuria which otherwise might not be suspected of this complication.

Biochemistry has contributed evidence of a physiologic relationship between the two diseases. Hypocalcemia is the most characteristic variation in milk fever, in which average blood calcium levels are reported as low as 5.13[3] and 3.31 mg. per 100 cc. of serum.[4] In milk fever, blood sugar tends to be elevated, while inorganic phosphorus and blood carbon dioxide capacity usually are reduced. Acetone levels in the blood and urine are within the normal range in uncomplicated milk fever. In typical acetonemia, however, blood calcium is normal, while sugar is reduced and acetone increased, resulting in the excretion of acetone in the urine in concentrations sufficient to react to the nitroprusside test. Blood acetone levels up to 6.0 mg. per 100 cc. of blood can be regarded as normal,[5] although lower levels may accompany reactions to the nitroprusside test.[6] Blood acetone values up to 100 mg. per 100 cc. of blood have been reported in cases of uncomplicated acetonemia with acetone as high as 1.3 Gm. per 100 cc. of urine.

Cases of milk fever complicated by acetonemia manifest the biochemical changes of both diseases, but they are usually less marked than in the uncomplicated forms. Blood calcium averages ranged from 6.51 to 8.88 mg. per 100 cc. while blood acetone did not exceed 44 mg. per 100 cc. and urine concentrations were proportionately lower than in uncomplicated acetonemia.[7] Evidently, the physiologic changes of these two diseases tend to be mutually antagonistic; or to apply the interpretation suggested in the alkalosis concept, ketosis springs from a physiologic attempt to prevent hypocalcemia.[2] When this mechanism is only partly successful, complicated milk fever results; complete prevention of hypocalcemia may lead to typical acetonemia.

PROCEDURES

This study consists in clinical observations and the results of examining specimens collected in general practice. Since the symptoms of milk fever are well defined, there was little question of the diagnosis. Blood specimens were collected prior to injection of calcium gluconate and again on the following day at approximately the same time. The blood was allowed to clot and the separated serum examined for calcium, according to the Tisdall method.[7] Urine was examined for acetone bodies following Rothera's nitroprusside procedure.[8]

The calcium injected in these cases was a home-prepared solution containing 25 Gm. of calcium gluconate and 2.5 Gm. of boric acid per 100 cc. The usual dose was 500 cc., although this was varied according to the size of the cow and her reactions to the injection. Supplementary therapy consisted in oral administration of Chlor-Ethamine (ethylenediamine dihydrochloride), a potent biological acidifier which breaks down in the intestines

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Presented before the Section on General Practice, Eighty-third Annual Meeting, American Veterinary Medical Association, Boston, Mass., Aug. 18-22, 1946.

Chlor-Ethamine (a brand of ethylenediamine dihydrochloride) was supplied for this study by Pitman-Moore Company, Indianapolis, Ind.

to yield 54.9 per cent of the dose as hydrochloric acid. It was administered as a drench, usually in a quart of water. To cows weighing less than 1,000 lb., we gave 2 heaping tablespoonfuls (40 Gm.) every eight hours, beginning as soon as the cow had recovered sufficiently following the injection of calcium gluconate. To heavier cows, we gave 3 heaping tablespoonfuls at the same intervals. Naturally, most of the medicating was entrusted to the owner of the cow.

RESULTS

Calcium Therapy.—In a series of 12 cases of milk fever, there were 6 which showed acetonuria, 5 without acetonuria, and 1 not tested for acetone in the urine. Upon first examining each case, a preliminary blood specimen was taken, following which a solution of calcium gluconate was injected intravenously, usually 500 cc. of a 25 per cent solution. Every case responded promptly, recovering consciousness and rising to their feet. As indicated in table 1, 6 cases in this series suffered a relapse within twenty-four to forty-eight hours after the initial calcium injection and required a second dose of calcium gluconate.

Cows 1 to 4 represent milk fever uncomplicated by ketosis, as indicated by absence of a positive nitroprusside test on the urine. They all received approximately the same dose of calcium and were examined for blood calcium level at approximately twenty-four hours after the injection. Average blood calcium findings indicate a preliminary level of 5.35 mg. per 100 cc. which by twenty-four hours later had increased to 5.72 mg. per 100 cc. for an average increase of 0.37 mg. per 100 cc. Cow 5 is omitted for lack of urine examination for acetone, and cow 6, because no blood specimen was collected twenty-four hours after the initial injection of calcium gluconate.

Cows 7 to 12 represent milk fever with ketosis. They received the same therapy as the preceding cases, and blood specimens were collected at approximately the same interval. In this group, the average preliminary blood calcium level was 6.07 mg. per 100 cc., which was increased to 7.70 mg. per 100 cc. twenty-four hours later, an average increase of 1.63 mg. per 100 cc.

For the entire series of 12 cases, including case 6, the average preliminary blood calcium level was 5.53 mg. per 100 cc., which had been increased to 6.81 mg. per 100 cc. by twenty-four hours after the injection of calcium gluconate, for an average increase of 1.27 mg. per 100 cc. In this series, there were 6 cases which relapsed, but all recovered eventually, except case 12, which may have choked to death in her stanchion when she went down the second time. Some of the cases required repeated treatment before they recovered completely.

Supplemented Calcium Therapy.—A second series of 10 cases of milk fever received the same dose of calcium gluconate as the preceding series, and they were managed in exactly the same manner with the exception that Chlor-Ethamine was administered orally during the 24-hour period following the injection of calcium gluconate. Only 1 case in this series was complicated by acetonuria (cow 21). Doses of the drug ranged from 75 to 285 mg. per kilogram, given as a single dose or divided into two or three (table 2). None of this series required a second injection of calcium gluconate, and only 1 (cow 22) manifested symptoms of partial relapse (*i. e.*, dullness, slight incoördination): she recovered spontaneously upon continuing the administration.

The 9 cases without ketosis (cows 22 to 30) showed an average preliminary blood calcium level of 4.66 mg. per 100 cc., which had increased to 7.01 mg. per 100 cc. after twenty-four hours, for an average increase of 2.33 mg. per 100 cc. In the single case with acetonuria (cow 21), the same increase was observed, 2.3 mg. per 100 cc. of urine, so that the average increase for the entire series was 2.33 mg. per 100 cc. Constipation was entirely absent in the cows receiving the treatment.

Chlor-Ethamine Alone.—One case of milk fever was treated during the initial 24-hour period with the drug alone. Two days after normal parturition, a 1,000-lb. grade Guernsey was found down with symptoms of milk fever. Blood examination at this time revealed a calcium level of 4.3 mg. per 100 cc. of serum. The cow was sufficiently conscious to be drenched, so she was given at once an oral dose of 2 heaping tablespoonfuls (40 Gm.). This dose was repeated seven hours, and again fourteen hours, after the initial dose. On the following day, twenty-four hours after the initial dose, the cow was still down but improved in general appearance and nervous reactions. Blood calcium at this time was 6.1 mg. per 100 cc., and 500 cc. of the 25 per cent solution of calcium gluconate was administered intravenously. The cow rose to her feet apparently normal two hours after the injection, and she remained normal. Dosing with Chlor-Ethamine was continued at eight-hour intervals. The following day, forty-eight hours after the first examination, blood calcium was 9.4 mg. per 100 cc. of serum, and the cow appeared completely recovered.

Chlor-Ethamine in Other Diseases.—In a few cases, we have administered the drug, either alone or in combination with intravenous calcium gluconate, where blood analysis proved the assumption of hypocalcemia incorrect. Cows showing mild symptoms suggesting incipient milk fever, which later proved to have been misleading, were dosed. In no case was there any indication that the treatment was harmful or detrimental to recovery from their disease. Nor did it appear beneficial, except perhaps to improve appetite and counteract constipation. In the majority of these cases, moreover, blood calcium was reduced after twenty-four hours of dosing with Chlor-Ethamine. As cases of ketosis are not included in this group, they will be reported separately.

DISCUSSION

Significance of Hypocalcemia in Milk Fever.—While hypocalcemia existed in all these cases of milk fever when they received their first injection of calcium gluconate, it is evident from a study of table 1 that some of the cows were laboring under a considerably reduced blood calcium level twenty-four hours later, even though they appeared completely normal. Cow 3, for example, was paralyzed and comatose when she received an injection of calcium; her blood calcium level was 5.3 mg. per 100 cc. On the following day, she appeared normal, notwithstanding a blood calcium level of 5.2 mg. per 100 cc., and she continued normal. This case, and others like it in these series, indicate that the symptoms of milk fever are not due to hypocalcemia. It seems more rational to regard hypocalcemia as another symptom, like paralysis and coma, a result of the underlying disturbance,— a symptom of secondary importance.

Blood Calcium Increase in Twenty-four Hours.—It was demonstrated in a previous report that an intravenous injection of calcium salts, in the dose range employed therapeutically, exerts an effect on blood calcium level which is so transient that by twenty-four hours after the injection blood calcium has been restored to its original level. The average 24-hour difference in blood calcium in 16 experiments was —0.08 mg. per 100 cc.[3] Any significant increase in blood calcium during the twenty-four hours following an injection of calcium salts, therefore, may be attributed to causes other than the addition of calcium to the blood stream.

It should be remembered, in judging the effects of intravenous calcium salts, that these injections exert at least three therapeutic actions: (1) add calcium to the circulating blood; (2) acidify, because of the acidity of the solution (pH 5 to 6); and (3) dilute and augment the volume of blood in the peripheral circulation. All these effects undoubtedly are beneficial in the clinical treatment of milk fever. The chief objections center about their brief duration and the danger of toxicity from hypercalcemia.

We have no data on blood calcium variations in cases of milk fever receiving no treatment whatsoever. The fact that intravenous calcium therapy in the 4 cases (table 1) uncomplicated by ketosis was

TABLE I—Intravenous Calcium Gluconate in Milk Fever

Case	Diagnosis[1]	Injection[2]	Interval[3]	Serum Ca mg. per 100 cc.[4] A	B	Diff.	Acetone[5] Urine A	B	Relapse[6]
1	Milk fever	23	23	5.5	6.3	+0.8	—	—	R
2	Milk fever	26	21	6.3	5.9	—0.4	—	—	R
3	Milk fever	27	25	5.3	5.2	—0.1	—	—	
4	Milk fever	25	24	4.3	5.5	+1.2	—	—	
5*	Incipient milk fever	12	19	3.6	5.3	+1.7			
6	Milk fever	18	50†	5.0	7.3	+2.3	—	—	R†
7	Incipient milk fever	20	24	7.8	8.7	+0.9	+	+	
8	Incipient milk fever	25	21	7.7	8.2	+0.5	+	+	R
9	Milk fever	18	25	6.8	9.0	+2.2	+	+	
10	Milk fever	25	23	3.5	7.5	+4.0	+	+	R
11	Milk fever	15	24	7.7	8.5	+0.8	+	+	
12	Milk fever	28	24	2.9	4.3	+1.4	+	+	R

*Specimens from case 5 submitted by Dr. W. C. Glenney, Elgin, Ill.

†Relapsed and received injections of calcium gluconate at twenty-four and forty-eight hours after initial injection (twice relapsed); no blood specimen at twenty-four hours, but specimen at forty-eight hours.

[1]All were cows that had calved within forty-eight hours preceding examination; cases classed as "milk fever" were paralysed (*i. e.*, down); "incipient milk fever" indicates preliminary symptoms (*e. g.*, depression, incoördination), but not paralysis. [2]Dose of calcium gluconate, expressed as Ca, mg. per kilogram; usually was 500 cc. of a 25 per cent solution. [3]Hours elapsed between injection of calcium gluconate and second (B) blood sample. [4]Blood calcium level (serum) determined immediately before injecting calcium gluconate (A) and again on the following day (B). [5]Reaction of urine to the qualitative nitroprusside test for acetone, before injecting calcium (A), and again on the following day (B). [6]Cases indicated by R in this column suffered relapse of the acute symptoms and required a second injection of calcium gluconate; other cases did not relapse.

followed twenty-four hours later by an average rise in blood calcium of 0.37 mg. per 100 cc. suggests merely the beginning of recovery from the physiologic disturbance of milk fever, despite clinical improvement in the case. The same therapy in similar cases, but complicated by ketosis, manifested blood calcium increases averaging 1.63 mg. per 100 cc. Addition of Chlor-Ethamine to this treatment was accompanied by still further increase in blood calcium level during the same period, 2.33 mg. per 100 cc.

Influence of Ketosis.—It is generally recognized that in cases of milk fever complicated with ketosis, the acute symptoms are not so prominent as in uncomplicated milk fever. Nor is the degree of hypocalcemia so marked. The latter difference is well illustrated in these cases. Preliminary blood calcium levels for the 14 cases without acetonuria averaged 4.87 mg. per 100 cc., whereas the 7 cases showing acetone averaged 6.01 mg. per 100 cc. Evidently, ketosis is associated with a mitigating influence on the tendency of the parturient cow to develop acute hypocalcemia.

The results in table 1 indicate further that ketosis may be associated with a more rapid recovery from the acute hypocalcemia than otherwise, for the average increase in blood calcium at twenty-four hours following intravenous calcium was four times as great in cases with ketosis (1.63 mg. per 100 cc.) as in uncomplicated cases (0.37 mg. per 100 cc.). Obviously, the number of cases examined is too small to permit more than a general comparison, but the difference between the results in these two groups of animals seems definite. Presumably, the underlying cause of hypocalcemia was counteracted more efficiently in the cases showing acetonuria than in the others. In terms of the alkalosis concept, acetonuria indicates an accelerated biologic attempt to counteract alkalosis by the elaboration of metabolic acids. It is interesting to note the same instance of relapse among the acetonuria cows as among the uncomplicated cases.

Effects of Chlor-Ethamine.—Oral administration to a comparable series of milk fever cases was with the hope that the hypercalcemic action of this acidifier, demonstrated in normal parturient cows,[7] could be utilized toward the relief of milk fever. Intravenous therapy seems desirable in the prostrate cow. By injecting calcium salts, a prompt, though transient, acidification is effected, together with the increase in blood calcium by direct addition. Following recovery of consciousness, Chlor-Ethamine may be administered conveniently and safely to assure maintenance of the acidifying action through sustained intestinal absorption of this acidifier.

The results of this study indicate: (1) virtually complete absence of relapse; and (2) sustained increase in blood calcium levels. There was partial relapse in 1 case (cow 22) which had received the smallest dose of Chlor-Ethamine, but which recovered without a second dose of calcium gluconate although she continued to receive Chlor-Ethamine. Blood calcium

TABLE 2—Intravenous Calcium Gluconate in Milk Fever Supplemented by Chlor-Ethamine

Case	Diagnosis	Calcium injection	Chlor-[1] Ethamine	Interval	Serum Ca mg. per 100 cc. A	B	Diff.	Acetone Urine A	B	Relapse[2]
21	Milk fever	21	80 (1)	20	5.7	8.0	+2.3	+	+	
22	Milk fever	25	75 (2)	31	3.8	4.5	+0.7	—	—	P
23	Milk fever	27	150 (3)	24	4.2	5.2	+1.0	—	—	
24	Milk fever	18	285 (3)	24	6.9	8.4	+1.5	—	—	
25	Milk fever	25	180 (2)	24	4.6	8.2	+3.6	—	—	
26	Milk fever	28	200 (2)	32	7.3	7.0	−0.3	—	—	
27	Milk fever	25	265 (3)	24	6.1	9.4	+3.3	—	—	
28	Milk fever	25	135 (3)	24	3.4	7.1	+3.7	—	—	
29	Milk fever	23	250 (2)	24	2.9	7.5	+4.6	—	—	
30	Milk fever	23	250 (2)	24	2.9	5.8	+2.9	—	—	

[1]Dose of Chlor-Ethamine administered orally as a drench, expressed as mg. per kilogram daily dose, the figure in parentheses indicating number of fractional doses *per diem*; fractions of daily dose were administered at regular intervals beginning when the cow had recovered sufficiently from the paralysis of milk fever to rise to her feet. [2]One instance of partial relapse (P), indicated by incoördination, but the cow recovered without a second injection of calcium gluconate; administrations of Chlor-Ethamine were continued.

twenty-four hours after injection of calcium gluconate and drenching with the drug had increased an average of 2.33 mg. per 100 cc., as compared to 0.37 mg. per 100 cc. in the 4 uncomplicated cases which received calcium alone. This represents a sixfold increase in effect on blood calcium level. Compared with the ketosis-complicated series, which revealed an average blood calcium increase of 1.38 mg. per 100 cc., Chlor-Ethamine was 70 per cent more effective in these cases than the physiologic compensatory mechanism.

These observations suggest that not only is Chlor-Ethamine, which in the body releases hydrochloric acid, effective in counteracting alkalosis, but it appears to be more effective for this purpose than the organic acids produced by the body. This is in accord with well-known principles of chemistry. A corollary to this observation would imply that the drug should be superior to chloral hydrate in the treatment of ketosis, assuming that chloral acts as an organic acidifier, after conversion in the body to urochloralic acid, or an intermediate. The use of Chlor-Ethamine in uncomplicated ketosis is under study.

CONCLUSIONS

1) While hypocalcemia is a characteristic symptom of milk fever, it is evidently not the fundamental physiologic defect, but appears to be secondary to a parturient alkalosis.

2) The coexistence of acetonemia, or ketosis, may be regarded as a result of a biological attempt to correct parturient alkalosis through neutralization of excess alkali. Thus, the hypocalcemic effects of parturient alkalosis may be diminished or completely nullified.

3) Artificial acidifying, employed as a substitute for, or supplement to, biologic neutralization of parturient alkalosis appears to be an effective means for improving the results of milk fever therapy. The therapeutic action of intravenous calcium solutions may be attributed to effects other than simply adding calcium molecules to the blood, such as acidifying and volume addition.

4) A procedure found successful in preventing relapse from milk fever was: (1) inject intravenously the standard dose of calcium solution, and allow time for the cow to regain consciousness and equilibrium; (2) with the cow on her feet, give the first dose of Chlor-Ethamine and repeat at eight-hour intervals as long as needed, usually twenty-four to forty-eight hours. The dose of the drug evolved from these studies is: for cows weighing less than 1,000 lb., 2 heaping tablespoonfuls (40 Gm.); for heavier cows, 3 heaping tablespoonfuls (60 Gm.), each dose dissolved in a quart of tap water and administered as a drench.

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The Resistance of Chronic Staphylococcic Bovine Mastitis to Massive Penicillin Therapy

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IN THE STATE of Washington, our studies have shown chronic mastitis to yield staphylococci more than twice as frequently as streptococci. Study, therefore, has been directed toward the control of staphylococcic mastitis.

The response of most staphylococcic infections to penicillin therapy,[1] together with the excellent results obtained in the treatment of mastitis due to *Streptococcus agalactiae*,[2, 3] has led to the general use of penicillin in the treatment of staphylococcic mastitis.

In our treatment of acute staphylococcic mastitis, infusions of penicillin usually have yielded satisfactory clinical results and frequently have been followed by apparent recoveries. However, following the infusion of from 25,000 to 400,000 Oxford units of penicillin into quarters infected with staphylococci, we have observed that, in an appreciable percentage of cases, the milk remained free from staphylococci for only a short time and that the plate counts tended to return to the previous levels. Previously, Slanetz and Allen[4] had reported that "staphylococcic mastitis is more difficult to cure and may require one or more treatments with 200,000 Oxford units per 100-cc. dose." Klein, Crisman, and Moor[5] failed, with 22,800-unit doses, repeated four or eight times, to eliminate staphylococci from five of seven quarters. Walker[7] infused penicillin in peanut oil containing 3 per cent beeswax with greater success. Little and coworkers,[7] in the Report of Animal Health, National Research Council, conclude: "A few research workers have claimed cures against chronic staphylococcal mastitis, but the majority of reports show such infection to be exceedingly difficult to eliminate from the udder."

While the number of acute cases of staphylococcic mastitis treated with penicillin and followed bacteriologically in our studies is too small to justify a positive statement, there appears to be a definite correlation between the extent of the fibrotic areas in the quarter, the length of time the infection has existed, and the persistence of the infection.

EXPERIMENTAL

Nine cows, with one or more quarters infected with long standing, chronic staphylococcic mastitis, were selected from the college herd. In every instance, the staphylococci were hemolytic when grown on the surface of 5 per cent bovine blood agar. In addition, they grew rapidly and utilized mannitol in Difco phenol red mannitol agar to which 70 Gm. of NaCl per liter had been added, giving a final salt concentration of 7.5 per cent. This medium, suggested by Chapman,[8] appears to be quite selective for pathogenic staphylococci, and only those colonies which grew readily on this medium and utilized mannitol, in addition to hemolyzing cow's blood readily, were considered as pathogenic.

Cow 215, Holstein-Friesian.—This cow, born December, 1939, last calved November, 1945, has completed her fourth lactation; at present, there are distinct fibrotic lobulations in every quarter. Low grade, chronic staphylococcic mastitis was diagnosed during her second period of lactation.

During 1945, plate counts of individual quarters usually ranged between 10,000 and 500,000 organisms per cubic centimeter, with *Staphylococcus aureus* always predominating, although corynebacteria, at times, were present in fairly high numbers. Various mixtures of sulfanilamide, sulfathiazole, sulfadiazine, and other experimental sulfonamide products were injected during 1945, with only temporary reductions in the bacterial counts.

An autogenous, formolized, alum-precipitated bacterin was injected subcutaneously on December 29, and the distinct negative phase produced is shown by the significant increase in organisms in the milk drawn forty-eight hours later (table 1).

The anticipated active immunity failed to control the infection, and the plate counts between January 6 and 19 were not signifi-

Published as Scientific Paper No. 686, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman.

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cantly different to those before the administration of the bacterin. The strip-cup readings became positive and, immediately following sampling and complete milking on the morning of January 26, 50,000 Oxford units of veterinary-grade penicillin were infused into each quarter; the evening milking was omitted, allowing the penicillin to remain in the udder for twenty-four hours. Cow 215 had not previously received penicillin.

Blood-agar plates of milk drawn the morning of January 27 revealed a curious phenomenon. All plates containing 1 cc. of milk failed to develop colonies, while the 1 : 10 dilutions from the left rear and right rear quarters gave seven and 12 colonies respectively on their plates. The 1 : 100 dilutions were without colonies. It is possible that a few organisms remained dormant and resistant during the time they were exposed to penicillin and then recovered in the presence of the blood agar. The absence of any growth on the plates containing 1 cc. of the milk might be accounted for by the presence of sufficient penicillin in that amount of milk to restrain growth in the milk-blood-agar mixture, while the smaller amount in the 1 : 10 dilution allowed growth.

All dilutions from all quarters were negative forty-eight and seventy-two hours following the penicillin infusions, but five days after treatment the plate counts on blood agar ranged from 167 to 765 and on mannitol salt agar between 27 and 350. The count steadily increased so that, on February 5, which was ten days after the penicillin infusion, the plate counts were between 5,000 and 63,300.

On February 9, 1 cc. of an autogenous staphylococcic toxoid bacterin was injected intradermally and, immediately after milking, 100,000 units of veterinary-grade penicillin were infused into each quarter. Plate counts two days later ranged from 0 to 96 organisms per cubic centimeter of milk and, one week later, ranged from 188 to 1,800 on blood agar and from 23 to 1,280 on salt mannitol agar. The counts soon rose higher than their previous levels.

On March 13, 100,000 units of penicillin in 20 cc. of water which contained 1 cc. of Triton (x100)* concentrate, giving a surface tension of approximately 30 dynes per centimeter, were infused into the right rear quarter. Triton (x100) is a surface-active synthetic employed in the dyeing industries to carry chromogens into fabrics. It was hoped that the presence of this product might carry the penicillin into the periphery of the mammary gland, assist in the penetration of the lesions harboring the staphylococci, and thus increase the efficiency of the penicillin. The following day, the injected quarter was somewhat tender, and the milk contained a small amount of catarrhal exudate, indicating ir-

*Supplied by Rohn and Haas Company, Philadelphia, Pa.

TABLE I—Plate Counts of Cow 215 Before and After Penicillin and Bacterin Therapies

Date	5% bovine blood agar				Salt mannitol agar			
1945	L.F.	R.F.	L.R.	R.R.	L.F.	R.F.	L.R.	R.R.
May 30	9,600	106,000	17,300	14,700				
Dec. 27	48,000	99,000	650,000	264,000				
Dec. 29	73,000[a]	68,000	290,000	314,000				
Dec. 31	330,000	1,115,000	2,000,000	1,151,000				
1946								
Jan. 6	51,000	71,000	1,390,000	520,000				
Jan. 12	37,700	172,000	168,000	200,000				
Jan. 19	76,000	64,000	272,000	226,000	48,000	39,000	160,000	178,000
Jan. 26	273,000[b]	410,000[b]	310,000[b]	340,000[b]				
Jan. 27	0	0	70	120	<10	<10	<10	<10
Jan. 28	<10	<10	<10	<10	<10	<10	<10	<10
Jan. 29	<10	<10	<10	<10	<10	<10	<10	<10
Jan. 31	199	167	765	700	27	62	210	350
Feb. 2	580	4,900	41	9,900				
Feb. 5	5,000	63,300	9,300	26,000				
Feb. 9	2,300[c]	4,800[c]	12,000[c]	9,600[c]				
Feb. 11	96	49	0	12				
Feb. 16	1,100	1,400	188	1,800	860	1,280	23	1,270
Feb. 21	20,500	25,600	32,000	440,000	11,200	10,000	20,400	19,600
Mar. 5	91,200	28,000	90,000	36,800				
Mar. 6					80,000	27,000	48,000	27,200
Mar. 7	22,000	280,000	260,000	320,000				
Mar. 8					21,000	240,000	220,000	290,000
Mar. 13	112,000	115,000	80,000	55,000[d]	157,000	93,000	42,000	37,000[d]
Mar. 14	50,000	900	300,000	1,200	49,000	200	48,000	800
Mar. 16	12,400	22,000	22,000	7,700	10,000	18,000	2,000	6,000
Mar. 19	18,500	17,900	130,800	64,700	17,700	15,100	13,100	48,300
Mar. 23	18,800	20,300	24,300	55,800	17,400	20,100	6,500	45,800
Mar. 30	13,600	15,200	21,100	10,500	6,300	9,600	14,000	6,000
Apr. 1	12,100	7,700	39,300	29,000	7,600	3,100	10,300	20,600
Apr. 3	15,100	10,000	40,000	41,400	7,600	4,500	5,700	23,900
Apr. 5	7,900	7,700	33,600	32,400	2,900	6,200	10,500	24,000
Apr. 30	9,600	106,000	17,300	14,700				

[a] Autogenous toxoid bacterin injected subcutaneously. [b] After milking, 50,000 units penicillin infused. [c] After milking autogenous toxoid bacterin injected intradermally and 100,000 units penicillin infused. [d] 100,000 units penicillin in Triton x100 infused.

ritation from the Triton. The reduction in the number of staphylococci, while only transient, was slightly greater in the untreated right front than in the treated right rear. As is shown in table 1, the staphylococci persisted in the milk, and milking was discontinued on April 5 because of low production. Cow 215 is representative of cows of valuable blood lines and good past production, which have been unsuccessfully subjected to various treatments but are being retained in the hope of obtaining valuable calves. She presents a satisfactory appearance and today could be sold in many sales rings. If brought into a clean herd, she could introduce a penicillin-resistant strain of staphylococci.

Cow 229, Holstein-Friesian.—This cow, born Jan. 14, 1941, last calved Oct. 24, 1945, is now in her third lactation. She became infected during her second lactation period. On January 26, she received 100,000 units of penicillin, half being infused into the right rear quarter and the remainder divided equally between the other three quarters. As is shown in table 2, a marked, but temporary, reduction in the number of bacteria followed. Six days later, blood-agar plate counts of 66, 205, 0, and 27 were obtained, respectively, from the left front, right front, left rear, and right rear quarters. Eight days after the infusion, the counts were 1,400, 900, 2,400, and 3,100, which rose on February 5 to 16,000, 12,800, 12,400, and 16,400, or approximately the preinjection levels.

MULTIPLE, MASSIVE THERAPY

The failure to control staphylococcic infection by single infusions of 100,000 units of penicillin into each quarter of cow 215 and 100,000 units in a Triton solution in a single quarter, and 100,000 units in cow 229, suggested that repeated treatments should be tested. The plate counts of cow 229 are shown in table 2 and of cows 163, 188, 193, 197, 207, and 259 in tables 3 and 4. All quarters treated were infected with chronic, low-grade *Sta. aureus* infections. Only cow 229 in this series previously had received penicillin therapy (table 2).

The following quarters were selected for therapy: 163—left rear; 188—left rear; 193—right front; 197—left front; 207—left rear; 229—left front, right front, left rear, right rear; 259—right rear.

On April 13, immediately following the morning milking, 100,000 units of purified (1,510 units per mg.), fresh (expiration date September, 1947), human-grade penicillin,† dissolved in 20 cc. of pyrogen-free water furnished with the penicillin, were infused into each quarter under experiment. This therapy was repeated immediately after each milking for four successive milkings, so that each infected quarter under treatment received a total of 400,000 units divided between four infusions, given at twelve-hour intervals. The data, presented in tables 2, 3, and 4, show that while the plate counts, both on mannitol salt agar and on bovine blood agar, were reduced for a time, bacteriologic cures were not obtained, and that the plate counts

TABLE 2—Plate Counts of Cow 229 Before and After Penicillin Therapy

Date	5% bovine blood agar				Salt mannitol agar			
1946	L.F.	R.F.	L.R.	R.R.	L.F.	R.F.	L.R.	R.R.
Jan. 12	5,000	9,000	2,400	1,800				
Jan. 26	9,700[a]	24,000[a]	9,600[a]	23,900[b]				
Feb. 1	66	205	0	27				
Feb. 3	1,400	900	2,400	3,100				
Feb. 5	16,000	12,800	12,400	16,400				
Feb. 23	1,800	4,500	2,900	3,300	<100	300	<100	<100
Mar. 19	9,400	26,500	15,200	22,900	8,300	17,200	19,300	36,600
Mar. 20	24,400	23,400	26,600	22,600	12,500	12,400	11,500	12,600
Apr. 1	19,500	10,400	19,800	21,900	9,800	4,500	11,400	10,900
Apr. 3	7,400	48,000	19,400	23,300	3,200	35,400	11,200	14,000
Apr. 5	3,400	14,000	2,240	3,400	2,000	5,900	810	1,800
Apr. 13	2,600[c]	139,000[c]	1,700[c]	3,800[c]	2,000[c]	3,450[c]	400[c]	1,900[c]
Apr. 14					<10	<10	<10	<10
Apr. 16	<10	<10	<10	<10	<10	<10	<10	<10
Apr. 18	20	80	30	30	<10	20	<10	<10
Apr. 23	4,100	4,900	3,200	1,520	1,700	270	1,440	300
Apr. 30	16,400	3,900	4,100	4,000	15,200	3,300	5,000	2,400
May 4	6,000	2,150	5,700	6,500	8,200	3,010	7,100	6,200
May 11	20,100	3,000	6,700	7,100	13,300	1,800	4,300	5,700
June 14	6,100	4,100	6,500	7,300	1,740	13,000	1,960	2,800

[a] After milking, 16,600 units penicillin infused. [b] After milking, 50,000 units penicillin infused. [c] After milking, 100,000 units penicillin infused. This was repeated after four successive milkings.

†Credit is due the Commercial Solvents Corporation of Terre Haute, Ind., for supplying the penicillin used in this and other experiments.

TABLE 3—Blood Agar Plate Counts Before and After Infusion of 400,000 Units of Penicillin

Cow	Average Feb. & March	April 1	April 3	April 5	April 13	April 14	April 16	April 18	April 23	April 30	May 4	May 11	June 8 & 13
163 LF	2,100	830	1,080	450	220	<10	50	<10	<10	<10	10	229	10
RF	100	26,500			2,100	<10	3,050	60	<10	20	<10	2	80
LR*	4,900		31,000	16,400	2,100	<10	<10	4,400	60	18,000	21,000	20,800	6,400
188 LF	18,600				13,000	<10	22,000	19,200	33,600	15,800	13,000	44,000	
RF	13				2,300	<10	<10	40	60	32,000	19,500	217,000	
LR*	1,300,000		2,700,000	900,000	284,000	<10	21,000	5,300	1,800	56,800	3,500,000	3,100,000	
193 RF*	26,600	31,600	18,100	49,200	17,600	<10	20	2,110	1,580	3,570	4,300	25,700	163,000
LR	900			1,300	2,300	<10	20	2,720	270	40	<10	160	210
RR	100			400	700	<10	<10	30	480	1,100	600	3,100	330
197 LF*	240,000	57,000	93,200	42,800	32,000		20	1,100	18,000	51,300	68,000	65,000	10,600
RF	100				10		<10	<10	220	30	<10	10	400
LR	100				80		<10	30	<10	20	<10	186	70
RR	100				10		<10	<10	140	<10	<10	24	380
207 LF	100				310		<10	<10	230	620	140	890	
RF	100				140		<10	<10	200	470	140	825	
LR*	4,000	10,200	21,300	6,200	5,700		60	260	5,200	2,740	4,900	20,800	
RR	100				80		<10	<10	60	<10	<10	<10	
259 RF	100	200			10		<10	<10	<10	<10	<10	<10	40
LR	2,300	900			900		<10	<10	<10	<10	<10	40	20
RR*	9,600	21,000			28,000		290	7,760	39,400	14,600	13,000	10,900	4,000

*Treated.

TABLE 4—Mannitol Salt Agar Plate Counts Before and After Infusion of 400,000 Units of Penicillin

Cow	April 1	April 3	April 5	April 13	April 14	April 16	April 18	April 23	April 30	May 4	May 11	June 5 & 13
163 LF	1,900	720	270	170	<10	110	<10	<10	<10	<10	108	100
RF				1,450	40	2,190	<10	<10	<10	<10	0	30
LR*	15,000	15,000	8,600	8,000	<10	<10	930	40	18,000	13,000	14,200	3,000
188 LF				11,600	200	16,200	16,300	26,000	12,700	9,500	23,000	
RF				1,300	<10	<10	<10	10	25,000	17,600	187,000	
LR*		1,200,000	72,000	112,000	<10	11,000	4,200	600	14,900	3,000,000	2,370,000	
193 RF*	32,200		19,400	8,400	<10	<10	850	1,520	2,330	900	12,000	92,000
LR			700	1,400	<10	<10	1,030	180	<10	<10	40	40
RR			100	700	<10	<10	20	170	460	610	2,800	410
197 LF*		73,000	43,700	21,000	<10	40	220	15,000	58,500	40,800	36,000	3,600
207 LR*	4,700	11,600	2,300	1,700	<10	30	60	<10	2,890	2,700	16,400	
259 RR*	18,300			1,100	<10	170	7,400	300	8,700	7,600	7,900	17,000

*Treated.

tended to approach the previous levels. It was observed that some lightly infected quarters, which received no penicillin, were rendered free from staphylococci, as determined by plate counts of 1 : 10 dilutions for as long a period as the more heavily infected quarters which were treated.

The chloride content of the milk was determined by titrations with silver chloride solution and by electrical conductivity by means of a 1,000-cycle, alternating current bridge.[9] Both the chloride content of the milk from the affected quarters and the electrical conductivity were abnormally high and were neither increased nor decreased significantly during or following the course of the treatment. The penicillin infusions had no deleterious effect upon milk production.

SUMMARY AND CONCLUSIONS

The efficacy of massive, repeated infusions of penicillin in the treatment of bovine mastitis was tested on ten quarters of 7 cows chronically infected with *Staphylococcus aureus*. It was found that the intramammary infusion of a total of 400,000 units per quarter, given in four infusions of 100,000 units at approximately twelve-hour intervals after each of four successive milkings, was followed by only a temporary reduction in the number of staphylococci shed in the milk of the treated quarters. The plate counts gradually tended to approach their former levels.

The infusion of the large amounts of penicillin was not followed by any clinical evidence of irritation or increase in either the chloride content or electrical conductivity of the milk, nor was the milk yield significantly reduced during or following the series of infusions.

It should be emphasized that this series included only chronic staphylococcic infections of long standing and that the number of quarters studied is small. The data and conclusions do not necessarily apply to acute, staphylococcic mastitis which receives early and adequate treatment and certainly not to mastitis due to *Streptococcus agalactiae*.

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Vibrionic Abortion in Cattle

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VIBRIONIC abortion in cattle was first recognized by Theobold Smith and his associates[1-6] during the period 1918-1923. Subsequently, Schroeder,[7] Traum,[8] and Barger,[9] reported the finding of the causative organism, *Vibrio foetus*, in several of the fetuses which they examined. More recently, Plastridge and Williams[10] described a culture medium for growing and maintaining cultures of *V. foetus* and reported results obtained in several herds with an experimental agglutination test.

Owing to the interest and coöperation of veterinarians and owners of brucellosis-free herds, it has been possible to obtain further information on the diagnosis of vibrionic abortion, losses resulting from *V. foetus* infection, and the extent and spread of infection as indicated by the agglutination test. The results obtained to date are reported here.

EXPERIMENTAL

Source of Material.—The data presented are based on the findings on fetuses and blood samples submitted for examination from individual cows suspected of aborting from *V. foetus* infection, and on the results of agglutination tests made periodically on ten herds that have been under observation for periods of six months or longer.

Agglutination Antigen.—This was prepared as described in a previous report.[10]

Examination of Aborted Fetuses.—Stomach fluid was inoculated onto the surface of blood agar and 0.1-cc. amounts into tubes of a soft agar medium (meat infusion containing 1 per cent peptone and 0.3 per cent agar, and adjusted to pH 7.4). A film for microscopic examination was also prepared and stained with dilute carbol-fuchsin. The inoculated mediums were placed in jars containing 10 per cent carbon dioxide and examined after three and six days of incubation at 37 C. Colonies appearing on blood agar were identified by microscopic and cultural tests. The soft agar was examined for zones of growth characteristic of *V. foetus*.

RESULTS

Vibrio foetus was detected in 20 of 72 fetuses examined. As shown in table 1, 10 were positive by direct microscopic examination, 10 yielded colonies on blood agar that were identified as *V. foetus*, and in 17 the organism was detected by culturing in soft agar. While 6 fetuses were positive by all three methods, it is of interest that 1 was positive by the microscopic test only, 1 by culturing on blood agar only, and 5 by culturing in soft agar only.

TABLE 1—Detection of Vibrio foetus by Bacteriologic Methods

No. fetuses pos. by 1 or more methods	No. positive by		
	Microscopic exam.	Culturing	
		Blood agar	Soft agar
20	10	10	17

Age of Fetuses at Time of Abortion.—While vibrionic abortion seems to occur most frequently after the fourth month of pregnancy, *V. foetus* was isolated in one instance from a 60-day fetus and once from a 90-day fetus. Of the remaining 18 bacteriologically positive fetuses, 4 were aborted during the fourth month of pregnancy and 14 after the fourth month of pregnancy.

Agglutinin Titers of Serums from Aborting Cows.—Serums from 188 brucellosis-free cows that aborted have been tested against *V. foetus* agglutination antigen. Fetuses from 72 animals were also examined. The results are summarized in table 2.

The agglutinin titers of serums from the 20 cows that aborted fetuses in which *V. foetus* was detected ranged from less than

Presented before the Section on Research, Eighty-third Annual Meeting, American Veterinary Medical Association, Boston, Mass., Aug. 18-22, 1946.

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1 : 200 to 1 : 3,200, with 17 (85%) having titers of 1 : 400 or higher.

Of the serums from the 52 cows that aborted fetuses in which *V. foetus* was not found, 6 (12%) possessed titers of 1 : 400 or higher. No organism was found in 4 of the 6 fetuses, and the cultures from 2 were overgrown with bacteria regarded as saprophytes. Of the remaining 46 fetuses,

TABLE 2—Bacteriologic and Serologic Tests for Evidence of Vibrio foetus Infection in Aborting Cows

Bacteriologic tests	No. specimens	No. serums wth agglutinin titers of: less than 1:200	1 200	1 400	1 800	1 1,600	1 3,200
+	20	1	2	10	3	2	2
0	52	36	10	4	2	0	0
Not made	116	56	10	24	19	5	2
Total	188	93	22	38	24	7	4
	%	49	12		39		

Corynebacterium pyogenes was found in 6, group C streptococci in 2, group G streptococci in 1, coliform organisms in 2, *Pseudomonas aeruginosa* in 1, and saprophytic bacteria in 3.

Blood samples only were received from 116 aborting cows, making a total of 188 samples examined. Of these, 73 (39%) gave titers of 1 : 400 or higher.

The number of herds in which vibrionic abortion has been diagnosed by cultural methods is 18, and by serologic methods only, 29.

Interpretation of Agglutination Reactions.—In our first report,[10] a titer of 1 : 100 was regarded as suspicious and 1 : 200 or higher as positive. However, the more recent findings given in table 2 suggest that serums giving partial agglutination in dilutions up to, and including, 1 : 200 should be regarded as negative, those giving complete agglutination at the 1 : 200 level and partial at 1 : 400 as suspicious, and those giving complete agglutination in dilutions of 1 : 400 or higher as positive.

OBSERVATIONS ON TEN VIBRIO FOETUS INFECTED HERDS

Incidence of Vibrionic Abortion.—The annual abortion rate attributed to *V. foetus* in ten brucellosis-free herds is given in table 3. The incidence of abortion ranged from 4 per cent in herd K to 20 per cent in herd D. Of the 363 cows in the ten herds, 45 (12%) aborted.

Fetuses and blood samples were received from 17 of the aborting cows. In ten instances, both the cultural and serologic tests were positive; in two, the cultural tests were positive and the agglutination tests suspicious; in four, the cultural tests were negative and the agglutination tests positive; and in one, both tests were negative.

Blood samples only were received from 28 of the aborting cows. Five were negative, 2 suspicious, and 21 positive to the agglutination test. Two of the negative samples were collected five months after abortion, and two were from cows that reacted positively to tests made three months after abortion. One of the suspicious cows was negative and 1 positive to tests made three months after they aborted.

In general, the results indicated that 40 of the 45 abortions recorded can be attributed to *V. foetus*, that three were possibly

TABLE 3—Annual Abortion Rate in Vibrio foetus Infected Herds

Herd	No. animals	Aborted	
		No.	%
K	50	2	4
H	37	4	11
L	84	10	12
S	63	8	13
V	16	2	13
T	14	2	14
P	28	4	14
C	25	4	16
M	21	4	19
D	25	5	20
Total	363	45	12

due to *V. foetus*, and that two were probably due to some other cause.

Relation of Agglutination Reactions to Abortion.—Blood samples have been drawn from the ten brucellosis-free herds and tested against *V. foetus* antigen at intervals of three months over periods of from six months to three years.

The stage of the breeding cycle when suspicious and positive agglutination reactions occurred was compared with the subsequent calving record. The results are given in table 4.

TABLE 4—Relation of Agglutination Reactions to Abortion

No. animals	Agglutination reaction, months after conception		Aborted	
	0 to 4	4 to 9	No.	%
9	0	S	2	22
20	S	0	0	0
22	S	S	0	0
26	0	+	11	42
38	+	0	1	3
72	+	+	5	7

Two of 9 cows that gave a suspicious reaction for the first time during the last half of gestation aborted; *V. foetus* was isolated in one instance and, while the other fetus was not received, the serum from the cow concerned gave a titer of 1 : 1,600 three months later. None of the 20 cows that changed from suspicious to negative, and none of the 22 cows that reacted suspiciously during both the first and last half of gestation, aborted.

The highest abortion rate occurred among the group of 26 cows that became positive during the last half of gestation. Eleven (42%) of these animals aborted. Of 38 cows that reacted positively when open or during early gestation and then changed to negative by the fourth month of gestation, 1 aborted. The abortion rate was 7 per cent in the group of 72 cows in which positive reactions occurred previous to, or soon after, conception and persisted during the last half of gestation.

Incidence and Spread of Vibrio Foetus Infection as Indicated by the Agglutination Test.—The incidence of animals in the ten herds that reacted positively to the agglutination test at the time of the first test varied from 10 to 60 per cent. During intervals of from six months to three years following the initial test, the incidence decreased in six herds, increased in two herds, and remained about the same in two herds. Infection apparently disappeared entirely from two herds as the result of positive animals changing to negative and, to a lesser extent, to the sale of positive cows.

Both the incidence of animals found positive and the number of abortions were usually greatest about the time of the initial test. This suggests that the abortion rate tends to be highest soon after the introduction of infection. As previously mentioned, while animals may acquire infection (as indicated by a positive agglutination reaction) at any stage of the breeding cycle, the chances of abortion occurring are greatest in cows which acquire infection during the last half of gestation. Hence, following the introduction of infection in a herd, abortions would be expected among the cows in advanced pregnancy, whereas many of the susceptible cows that were open or recently bred might acquire infection and recover without aborting.

The findings on three representative herds are given in tables 5, 6, and 7.

Herd T.—Agglutination reactions given by animals in herd T are summarized in table 5. Two of the 7 cows found positive on the Oct. 16, 1944, test aborted: 1, one month before, and 1, one month after, the initial test. *Vibrio foetus* was isolated from 1 of the fetuses and the other was not examined. The 2 cows remained in the herd; both became negative, 1 after six months and 1 after one year, and both conceived and calved normally

TABLE 5—Results of Agglutination Tests in Herd T

Animals	10/16/44	3/23/45	5/1/45	10/13/45	5/15/46
No. Pos.	14	14	14	15	23
old	7(2a)	4	5	3	0
new		5	0	2	0
→0	1	4	2	4	
sold	2	0	0	1	
% pos.	50	64	36	33	0

a=Aborted.

following abortion. During the period from Nov. 17, 1944, to May 15, 1946, no further abortions occurred, and the incidence of positive cows decreased from 64 to 0 per cent. Three were sold and 11 changed from positive to negative. It is of interest that, of the 7 cows that developed positive reactions following the initial test, 4 became positive when open, 2 when two months pregnant, and 1 (previously positive) when five months pregnant.

Herd C.—This herd is of special interest because, during the year preceding the first blood test, 8 of 18 adult cows aborted. Six of the 8 had been calfhood vaccinated with *Brucella abortus* vaccine. At the time of the first test, the serums from 3 of the cows, including 1 vaccinated 3-year-old heifer, were positive to the agglutination test for brucellosis, 3 were positive with *V. foetus* antigen, and 2 (aborted nine and twelve months, respectively, before the test) reacted negatively to both antigens. The positive brucellosis animals were sold soon after the first test. The results of periodic tests made with *V. foetus* antigen are given in table 6.

TABLE 6—Results of Agglutination Tests in Herd C

Animals	12/6/44	2/11/45	8/30/45	12/16/45	5/20/46
No. pos.	24	29	27	27	25
old	11(3a)	9	14	10	5
new		7(1a)	3	2	2
→0	2	0	6	2	
sold	0	2	1	5	
% pos.	46	55	63	44	28

a=Aborted.

Three of the 11 animals found positive on the first test had aborted within a period of three months before the test was made. *Vibrio foetus* was isolated from 1 of the fetuses. One of the 7 animals that became positive by the time of the second test aborted.

During the following period of fifteen months, 7 animals became positive but none aborted. All were open or recently bred at the time they reacted positively. Five were 2-year-old heifers that had not been tested previously.

TABLE 7—Results of Agglutination Tests in Herd L

Animals	10/2/44	1/5/45	2/10/45	3/29/45	5/21/45
No. pos.	84	88	83	83	83
old	40(4a)	29	30	32	31(4a)
new		9	9	2	10(2a)
→0	8	7	5	1	
sold	3	1	2	2	
% pos.	48	43	47	41	49

a=Aborted.

During the 17-month period of observation, 14 animals became positive, 10 changed from positive to negative, and 8 positive animals were sold, 4 because of sterility.

Herd L.—The results of tests made on this herd are recorded in table 7. Four of the cows found positive and 1 found negative on the initial test had aborted within a period of two months preceding the test. During the period from Oct. 2, 1944, to March 29, 1945, no abortions occurred. However, during the interval between the March 29, 1945, and May 21, 1945, tests, 4 cows that had been positive or suspicious from the start aborted. Two of 10 cows that reacted positively for the first time on the May 21, 1945, test aborted soon after the test was made. Both were heifers that appeared to have acquired infection during the last half of gestation.

The incidence of positive reacting cows remained between 41 and 49 per cent during the seven-month period of observation. Unfortunately, that herd was sold soon after the last test was made.

Relation of Vibrio Foetus Infection to Failure to Conceive.—The possibility that *V. foetus* may lower the conception rate, as well as cause abortion, is indicated by recent preliminary observations. The breeding records of 12 cows following vibrionic abortion are given in table 8.

Two of 8 cows that aborted and subsequently gave a negative agglutination reaction failed to conceive following six

TABLE 8—Breeding Record Following Vibrionic Abortion

No. cows	Agglutination reaction: Time of abortion	Agglutination reaction: 6 to 9 mo. after abortion	Breeding record: Sterile*	Aborted	Normal
8	+	0	2	0	6
4	+	+	1	2	1

*Failed to conceive after 6 to 8 services.

to eight services and were sold; 6 conceived and calved normally. Of 4 cows that continued to react positively following abortion, 1 failed to conceive, 2 aborted a second time, and 1 conceived and calved normally. The number of cows on which records are available following vibrionic abortion is small owing to the disposal of 10 aborters and to the need for longer periods of observation.

In five herds under observation, the owners have reported a lowered conception rate following the occurrence of vibrionic abortion. The possibility that infection with *V. foetus*, in the absence of abortion,

may contribute to this condition is being considered.

To date, the breeding records and agglutination reactions have been compared on 76 animals in four of the ten herds under observation. Of the 38 animals in the serologically negative group, 21 conceived following one service, 16 required from two to four services, and 1 failed to conceive and was sold. The average number of services required by the 37 animals that conceived was 1.8. In comparison, of 38 heifers and cows that reacted positively before they were bred, 9 conceived after one service, 18 after two to four services, 4 after five, 4 after six, and 3 were sold because of failure to conceive. The average number of services required, excluding the 3 animals that were sold, was 3.0.

Evidence of Infection in Bulls.—The cows in seven of the ten herds were bred naturally. In five of the herds, the bulls reacted negatively to the agglutination test. The 3 bulls used in herd L were tested once only. Their serum titers were 1 : 400, 1 : 800, and 1 : 1,600, respectively. The 3 bulls used in herd K were negative at the time of the first test; 1 remained negative during the two-year period of observation; and 2 gave titers of 1 : 800 and 1 : 1,600, respectively, twelve months after the initial tests were made. One was sold and the titer of the other one decreased from 1 : 1,600 to 1 : 400 during the following twelve months.

It appears from these findings that, if agglutinin titers of 1 : 400 or higher are accepted as evidence of infection, bulls must be regarded as susceptible to infection with *V. foetus*.

SUMMARY

1) Vibrionic abortion has been detected in 18 herds by bacteriologic examination of aborted fetuses and in 29 by serologic tests only.

2) Of 20 fetuses in which *Vibrio foetus* was found, 10 were positive by direct microscopic examination, 10 by culturing stomach fluid on blood agar, and 17 by culturing stomach fluid in meat infusion broth containing 0.3 per cent agar.

3) A comparison of the agglutinin titers (complete agglutination) of serums from the 20 cows aborting infected fetuses with those of 52 cows aborting fetuses in which *V. foetus* was not found suggests that titers of less than 1 : 200 should be classed as negative, 1 : 200 as suspicious, and 1 : 400 or higher as positive.

4) Observations on ten *V. foetus* infected herds are summarized as follows: (*a*) The annual abortion rate attributed to *V. foetus* ranged from 4 to 20 per cent with an average of 12 per cent. (*b*) The incidence of animals that reacted positively to the initial agglutination test ranged from 10 to 60 per cent. On subsequent tests, the incidence decreased in six herds, increased in two, and remained about the same in two. (*c*) Of 26 cows that became positive during the last half of gestation, 42 per cent aborted; of 38 cows that were positive and became negative by the fourth month of gestation, 3 per cent aborted; and of 72 cows positive during both the first and last half of gestation, 7 per cent aborted. (*d*) A lowered conception rate has been observed in five herds following the occurrence of vibrionic abortion. (*e*) Agglutinin titers of from 1 : 400 to 1 : 1,600 were observed in 5 of 6 bulls in two of the seven herds that were bred naturally.

DISCUSSION*

DR. A. B. CRAWFORD (Beltsville, Md.).— I would like to ask Dr. Plastridge what is the nature of his antigen.

DR. PLASTRIDGE.—The method of preparing the antigen was omitted because of lack of time. The medium used consists of meat infusion containing 1 per cent peptone and 0.3 per cent agar and is adjusted to pH 7.4. Ten-cc. amounts are sterilized in 1-in. by 6-in. tubes. The inoculum is prepared from seed cultures by suspending the cell-containing layer in each tube in 3 cc. of nutrient broth. Three tenths cc. of the suspension is deposited in the upper third of the medium in each tube used for antigen production. The inoculated tubes are incubated at 37 C. for six days in jars in which about 10 per cent of the air is replaced with carbon dioxide.

The resulting growth is suspended in saline containing 0.3 per cent formalin and removed with a pipette. The suspension is then diluted with three parts of formalinized saline and centrifuged at 1,600 r.p.m. for thirty minutes. The supernatant is removed, the sedimented agar resuspended and centrifuged, and the supernatant added to the original. The combined supernatants are placed in Lusteroid tubes and centrifuged at high speed. The deposited cells are resuspended in a small volume of formalinized saline and stored at 5 C. The stock antigen is diluted for use to a density of 1.5 on the McFarland nephelometer scale.

DR. HADLEIGH MARSH (Bozeman, Mont.).— In regard to this agglutination test for *Vibrio foetus*, we have been doing experimental work on *V. foetus* in sheep for a number of years.

*After the presentation of the paper, the following discussion was conducted.

I have one or two cattle cultures, too, but we have studied the organism in sheep. I do not know whether there is any difference in their reactions on culture medium, but we have been able to make an antigen in a much simpler manner. We have been growing the organism on serum agar in CO_2 atmosphere, and we have been able to prepare a very effective antigen that way. We make our own agar, but we don't use the ordinary dehydrated agar. It is a modification of the old agar, but we get very satisfactory results. The agar has to be of the right concentration. If it is a little too hard, it doesn't work. We have had cultures growing for a couple of hundred transfers on those serum agar slants. It is much easier to make an antigen from those cultures and has been very satisfactory.

DR. PLASTRIDGE.—Do you add the serum to the slanted slope of the agar, or add it to the melted agar?

DR. MARSH.—To the melted agar.

DR. PLASTRIDGE.—What concentration?

DR. MARSH.—About 10 per cent.

DR. PLASTRIDGE.—We haven't tried that out, but we are certainly going to.

DR. CRAWFORD.—Does this antigen you prepare result in positive reactions in the infected cows?

DR. PLASTRIDGE.—That is a matter that is very difficult to decide. If you can actually isolate Vibrio from the fetus, then you can say you know definitely the cow has an infection with this germ. If you test a cow and you do not have a positive reaction, you can never be sure whether you have infection or not.

I think that we have to be careful about interpreting agglutination reactions, because it seems fairly certain that titers of 1 : 200 or lower should not be regarded as indicative of infection. I do not know why, but cow serum does seem to give titers up around 1 : 100 or even 1 : 200. That is why it appears that, in interpreting the test, a serum regarded as positive should have a titer of at least 1 : 400, and I wouldn't say a titer of 1 : 400 always indicates infection. I suspect at times it does not. The test should be regarded as experimental; it isn't as refined as our Bang's test. It is just a start on the problem, but I do think it has a great deal of promise.

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Antigenic Relationship Between the Genus Mycobacterium and the Genus Corynebacterium in Goats and Cattle

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AN ANTIGENIC relationship exists between the species of bacteria within the genus Mycobacterium (Crawford[1]), (McCarter, Kane, Hastings[2]), (Dolgopol[3]). Canham[4] has suggested that a similar relationship extends to the genera within the family Mycobacteriacea and that infection with nonacidfast organisms of the family may result in the host animal becoming reactive to Mycobacterium products and vice versa. If this is true, it may offer an explanation as to why some cattle which react to johnin and/or tuberculin show no lesions of Johne's disease or tuberculosis on postmortem examination. Since several species of the genus Corynebacterium are pathogenic for both sheep and cattle, it seemed possible that some of the "no visible lesion" reactors to johnin and/or tuberculin might have been sensitized by organisms of this genus.

Schoening's[5] finding that about 30 per cent of normal horses had sufficient protective properties in their serum to protect guinea pigs against two minimum lethal doses of diphtheria toxin suggests that other species may carry such antibodies. Although we know of no published record of infection with *Corynebacterium pseudotuberculosis* being responsible for a reaction to either johnin or tuberculin, Dr. James W. Benner[6] stated in a personal communication that he had found lesions of caseous lymphadenitis in goats that reacted to johnin, and had isolated *C. pseudotuberculosis* from these lesions. However, organisms indistinguishable from *Mycobacterium paratuberculosis* were also found to be present in scrapings from the intestinal tracts of these goats.

The experiments described in this paper were planned to determine (1) whether goats injected with *C. pseudotuberculosis* would subsequently react to johnin or tuberculin, and (2) whether serums from cattle highly sensitive to allergens derived from species of Mycobacterium would neutralize the toxin of *Corynebacterium diphtheria*.

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PROCEDURE AND RESULTS WITH GOATS

A suspension of *C. pseudotuberculosis* was made by washing a two-week-old growth of organisms from an agar slant with 10 cc. of saline. Each of 2 goats, about 15 months old, were injected with 2 cc. of this material. One goat was inoculated intraperitoneally and 1 subcutaneously. The goats were tested intradermally with 0.2 cc. each of johnin and tuberculin at thirty-day intervals for one year. No reactions were obtained from either the johnin or tuberculin tests. During the observation, the goats were fed a ration of chopped grain and alfalfa hay.

PROCEDURE AND RESULTS WITH CATTLE

Serums from two groups of cattle were tested for substances positive against diphtheria toxin. Group 1 consisted of 39 cattle from this station, selected at random, that reacted to neither johnin nor tuberculin. Part of them were from the station dairy herd; the others were from a group that had been brought to the station from various parts of Alabama for use in the study of parasites. Group 2 consisted of 50 cattle divided into five equal lots. Each lot had been highly sensitized by intraperitoneal injections of a mineral-oil suspension of a different species of acidfast organism as follows: (1) *Mycobacterium tuberculosis avium*, (2) *Mycobacterium tuberculosis hominis*, (3) *Mycobacterium tuberculosis bovis*, (4) *Mycobacterium paratuberculosis*, and (5) *Mycobacterium phlei*.*

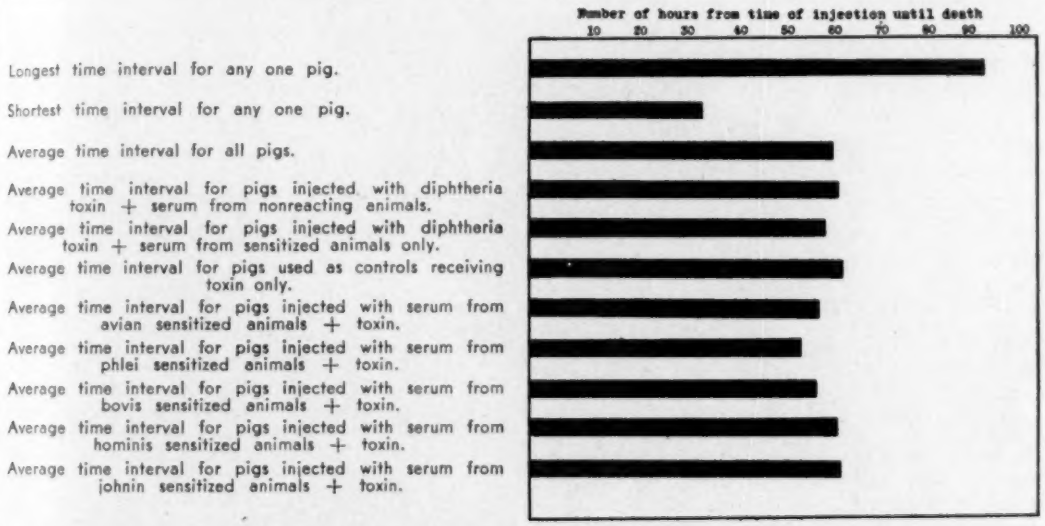
Ten cc. of blood were collected under aseptic conditions from each of the 89 animals, centrifuged, and the serum drawn off. One previously determined m.l.d. of diphtheria toxin

*The degree of sensitivity of these animals was determined at the time serum was taken from them by testing with a homologous testing material intradermally in the caudal fold. All animals gave a marked reaction to this test.

was mixed with 1 cc. of serum from each sample and the mixture allowed to stand at room temperature for one hour (Schoening[5]).

Each serum-toxin mixture was injected subcutaneously into a guinea pig, and the animal observed for its response. The m.l.d. of diphtheria toxin (Parke, Davis; Lederle) was established by determining the smallest amount of toxin which was invariably fatal to a 250-Gm. guinea pig within ninety-six hours after subcutaneous injection. The potency of the diphtheria toxin was checked by mixing 1 cc. of saline with 1 m.l.d. of toxin and injecting it subcutaneously into control guinea pigs at the time of the experiment. Ninety-six guinea pigs were used in all, 7 of which were controls.

The following bar graph shows the results of the guinea pig injections.


Number of hours from time of injection until death
10
20
30
40
50
60
70
80
90
100
Longest time interval for any one pig.
Shortest time interval for any one pig.
Average time interval for all pigs.
Average time interval for pigs injected with diphtheria toxin + serum from nonreacting animals.
Average time interval for pigs injected with diphtheria toxin + serum from sensitized animals only.
Average time interval for pigs used as controls receiving toxin only.
Average time interval for pigs injected with serum from avian sensitized animals + toxin.
Average time interval for pigs injected with serum from phlei sensitized animals + toxin.
Average time interval for pigs injected with serum from bovis sensitized animals + toxin.
Average time interval for pigs injected with serum from hominis sensitized animals + toxin.
Average time interval for pigs injected with serum from johnin sensitized animals + toxin.

Graph 1—Results of Simultaneous Guinea Pig Injections of Diphtheria Toxin and Bovine Serum

There does not appear to be any significant difference between the different groups of guinea pigs. In all cases, the animals were dying by the fortieth hour and continued to die through the eightieth hour.

DISCUSSION

It is believed that there was a combination of both Johne's disease and caseous lymphadenitis in the goat herd reported by Dr. Benner. The goats that were artificially inoculated with *C. pseudotuberculosis* showed no reactions at any time to johnin.

Since serum from either normal cattle or cattle that were highly sensitized by Mycobacterium showed no antitoxin against a toxin prepared from *C. diphtheria*, it is not probable that an allergen prepared from a species of Mycobacterium would elicit a response in an animal previously sensitized with a member of the genus Corynebacterium. However, the production of antitoxin and skin sensitization may be somewhat different phenomena, since Mycobacterium produces very little or no exotoxin but does produce skin sensitization.

SUMMARY

1) Goats injected with *Corynebacterium pseudotuberculosis* failed to react when tested with johnin and hominis tuberculin.

2) Serums from both apparently normal cattle and cattle highly sensitized to five Mycobacterium species failed to neutralize diphtheria toxin.

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Experimental Treatment of Trichostrongylosis in Sheep with Phenothiazine

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OBSERVATIONS extending over a period of years indicated that, in Montana sheep, the genus Trichostrongylus is probably responsible for more clinical parasitism than other intestinal Nematoda.

In the American literature on parasites of sheep, there are few reports of investigation of methods of treatment and control of parasitism due to worms of this genus. Habermann and Harwood[1] state that phenothiazine in a dose of 25 Gm. was partially effective against Trichostrongylus in mature sheep. Shorb, Habermann, and Heemstra[2] report that, in lambs 6 months old, a dose of 25 Gm. of phenothiazine reduced the egg count of Trichostrongylus by 81.6 per cent. In Australia, Gordon has worked with the control of trichostrongylosis. In 1939, Gordon[3] reported that fecal egg counts indicated that, in a dose of 0.6 Gm. per kilogram, phenothiazine was highly effective against Trichostrongylus. In 1940, Gordon and Whitten[4] reported that phenothiazine in a dose of 15 Gm. for lambs about 5 months old, weighing about 20 kg. (44 lb.), was highly efficient against Trichostrongylus. Gordon[5] found that phenothiazine was not effective against immature Trichostrongylus. In 1945, Gordon[6] recommended phenothiazine as about 90 per cent efficient in treatment of trichostrongylosis in doses of 16 Gm. for lambs 4 to 8 months old, 20 Gm. for sheep 12 to 18 months old, and 24 Gm. for mature sheep. He stated that, for efficiency against immature Trichostrongylus, it was necessary to double these doses. He also stated that a daily intake of 0.5 Gm. of phenothiazine will inhibit egg production, as well as prevent the development of larvae in feces. Joint Publication No. 4 of the Imperial Agricultural Bureaux[7] in England reports experimental treatment of lambs with phenothiazine for Trichostrongylus, in which the results were evaluated both by egg counts and by postmortem worm counts. The findings were that a 10-Gm. dose was not effective against *Trichostrongylus axei*, while a 20-Gm. dose was effective. Against the intestinal species of Trichostrongylus, a 30-Gm. dose was not effective, while 40- and 50-Gm. doses were partially effective.

In 1945, Seghetti and Marsh[8] reported results of phenothiazine treatment of ewes, in which the predominant nematodes were Trichostrongylus species. A dose of 25 Gm. for mature sheep was effective in immediate reduction of the egg count but was not effective in killing the worms, as shown by the fact that the egg counts increased after the initial reduction, under conditions unfavorable for reinfection. The results also indicated that an average consumption of 0.8 Gm. of phenothiazine per day with salt was effective in inhibiting egg production.

EXPERIMENTAL

In the fall of 1945, an opportunity presented itself to test the value of phenothiazine treatment of a flock of ram lambs which were heavily infected with Trichostrongylus spp. On Aug. 10, 1945, 117 ram lambs owned by the Montana Agricultural Experiment Station were moved from mountain summer range to an irrigated pasture at Bozeman, and were run in the same pasture with a small bunch of ram lambs which had been on irrigated pastures all summer. About September 15, these lambs began showing diarrhea and, on October 6, they were brought to the attention of the veterinary department. At that time, diarrhea was general and the lambs as a whole presented the picture of clinical intestinal parasitism. Fecal samples indicated Trichostrongylus infection as the cause. This diagnosis was confirmed by autopsy of 2 lambs which were destroyed because of their very weak condition.

The lambs were then moved into the corral and placed on dry feed; they were started on alfalfa hay and oats. When they reached full feed they were receiving 2 lb. of oats and alfalfa *ad libitum*. Experience had shown that, when lambs suffering from trichostrongylosis are removed from the infective pasture and fed hay and grain, they may recover without treatment. It was decided to set up an experiment to determine whether it would pay to treat such lambs, in addition to placing them on feed. Eight of the 117 lambs were in bad condition and were removed from the flock to a hospital bunch. Three of these 8 lambs were killed, and the parasite counts for the abomasum and small intestine were as follows:

From the Montana Veterinary Research Laboratory (Montana Experiment Station and Livestock Sanitary Board coöperating). Paper No. 56, Journal Series, Agricultural Experiment Station, Montana State College, Bozeman.

The authors acknowledge the assistance of members of the staff of the Department of Animal Industry of the Montana Experiment Station in setting up this experiment and collecting the data.

A-2019

Abomasum .....15,000 Trichostrongylus spp.
Intestine .....48,900 Trichostrongylus spp.
Fecal egg count ....140 per gram (feces fluid).

A-3019

Abomasum .....7,000 Trichostrongylus spp.
Intestine .....31,000 Trichostrongylus spp.
400 Nematodirus spp.
Fecal egg count ....5,800 Trichostrongylus and
400 Nematodirus per gram.

C-5237

Abomasum .....1,200 Trichostrongylus spp.
Intestine .....18,200 Trichostrongylus spp.
560 Nematodirus spp.
Fecal egg count ....240 per gram (feces fluid).

The type of parasitism in this flock of sheep was further established by an autopsy of C-5137, which died of pneumonia on December 12, after the experiment had been in progress for six weeks. The worms present were as follows:

Abomasum .....7,800 *T. axei* and *T. colubriformis*.
300 *Ostertagia circumcincta*.
Small intestine .....22,800 *T. colubriformis*.
Fecal egg count .....1,360 per gram.

These lambs were free from Hemonchus, and the only other nematodes of importance that may have been present in small numbers were Ostertagia species. These worm counts confirmed the diagnosis of trichostrongylosis.

On October 26, the 109 lambs were divided at random into two lots of 55 and 54. Lot 1, consisting of 55 lambs, averaged 95.07 lb. in weight, while lot 2, consisting of 54 lambs, averaged 95.36 lb. in weight. Fecal samples had been taken from all the lambs on October 22, and egg counts per gram of feces were recorded. The eggs were recorded as "trichostrongyloid" and Nematodirus. The trichostrongyloid count may have included a small percentage of Ostertagia and *Chabertia ovina* in some individuals, but the trichostrongyloid count was determined to be principally Trichostrongylus spp. The average trichostrongyloid count for lot 1 was 1,760, ranging from 10 to 3,530, while for lot 2 it was 1,873, with a range of 465 to 4,440. The Nematodirus averages were 42 for lot 1 and 39 for lot 2. It will be noted that the two lots, set up at random, varied less than 1 lb. in average weight, and showed fecal egg counts which corresponded very closely.

Lot 1 was treated on October 26 with phenothiazine, while lot 2 was allowed to go without treatment. At the time of treatment, the lambs had recovered to some extent from the acute symptoms of parasitism. The dose of phenothiazine used was 37.5 Gm. in the form of three boluses, each containing 12.5 Gm. of phenothiazine. This dose was three times the standard dose for lambs and one and a half times the standard dose for mature sheep. While these lambs were only 6 months old, their size placed them close to that of average mature sheep. Previous experience had shown that the standard dose of phenothiazine would immediately reduce the fecal egg count of Trichostrongylus eggs to a very low figure, but that the egg count would gradually rise, with little or no opportunity for reinfection, until in thirty to sixty days the egg count would approach the pretreatment count.

The results of the treatment were measured by fecal egg counts made 13 days, 30 days, 69 days, and 90 days after treatment; and by weights taken 18 days, 30 days, 60 days, 90 days, and 120 days after treatment. The egg counts were made on 20 lambs from each lot, selected at random, the same 20 lambs being used for each post-treatment count. One of the 20 lambs from lot 2 died on December 12, and the original count for this lamb was not used in the calculations. The average trichostrongyloid egg count for the 19 lambs used as the sample of lot 1, on October 22, was 1,635, as compared with 1,760 for the entire lot. The average for the 20 lambs used as the sample of lot 2 was 2,011 on October 22, as compared with 1,873 for the entire lot.

On November 8, thirteen days after treatment, the average egg count for the 20 lambs sampled in lot 1 was only 70. Thirty days after treatment, the count for these lambs was again 70, and the average of counts made on three successive days, the 68th, 69th, and 70th after treatment, was 44. The last count was made ninety days after treatment and showed no significant change, the average being 100.

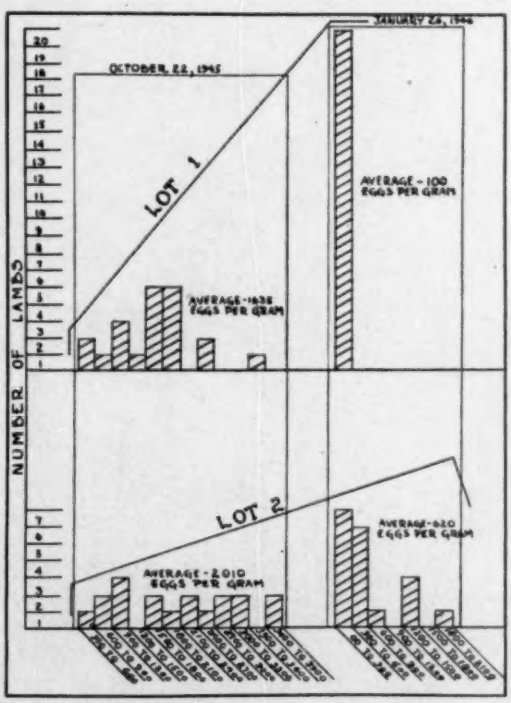
JANUARY 25, 1946
OCTOBER 22, 1945
LOT 1
AVERAGE - 100 EGGS PER GRAM
AVERAGE - 1635 EGGS PER GRAM
NUMBER OF LAMBS
LOT 2
AVERAGE - 2010 EGGS PER GRAM
AVERAGE - 620 EGGS PER GRAM

Fig. 1—Frequency histograms of fecal egg counts of the 20-lamb samples of the two lots of lambs before treatment and three months after treatment.

The control lambs, lot 2, showed a phenomenon which has been observed repeatedly at this laboratory when controlled tests of the effect of treatment have been set up in the autumn for a flock of lambs which have been showing clinical parasitism for some time. The average egg count on the 20 lambs sampled in lot 2 dropped to 419 on November 8, and continued to drop until November 26, when it was 116, which was not significantly different from the count for the treated lot. At sixty-nine and ninety days after treatment of lot 1, there was a marked difference in the average egg counts for the two lots. The counts for lot 2 rose to 603 and 617, as compared with 44 and 100 for lot 1.

The histograms in figure 1, showing the fecal egg count frequencies for the group of lambs sampled from each lot on Oct. 22, 1945, before treatment, and on Jan. 26, 1946, give information which supports the conclusion drawn from the averages. The lambs constituting the lot samples were selected at random, and there was a much wider distribution around the mean in lot 2 than in lot 1, with a higher average. Although this discrepancy existed in the original frequencies, the distribution shown in the histograms of the frequencies for the January 26 counts indicates a significant difference between the two lots due to the treatment. In lot 1, all the counts were below 300 per gram, while in lot 2 only 7 of the 19 counted were in this classification, and five counts were above 1,200 per gram. Of the 5 lambs showing counts above 1,200 on January 26, only 1 was in the high count classification on October 22.

The effect of treatment with phenothiazine was also measured by comparing the weights of the two lots at 30, 60, 90, and 120 days after treatment. The average weights for the two lots on October 26, when the phenothiazine was administered to lot 1, were almost identical, lot 1 weighing 95.07 lb., and lot 2 weighing 95.36 lb. The averages for the two lots showed increasing divergence on the four weighing dates following treatment, the difference being 3 lb. on November 26, 6 lb. on December 26, 11 lb. on January 26, and 14.5 lb. on February 21, when the final weight averages were 147.8 lb. for lot 1 and 133.3 lb. for lot 2. The "t" test for statistical significance of the difference between the mean final weights of the two lots gives a value of 2.64 for "t," for 100 degrees of freedom, which indicates significance at the 1 per cent level. An examination of the distribution of the weights in each lot on February 21, as shown by the histograms of figure 2, shows that in lot 2 there were 7 sheep weighing less than 100 lb., while there were no sheep weighing less than 100 lb. in lot 1. In lot 2, there were 14 sheep weighing not more than 120 lb., while there was only 1 sheep in this category in lot 1.

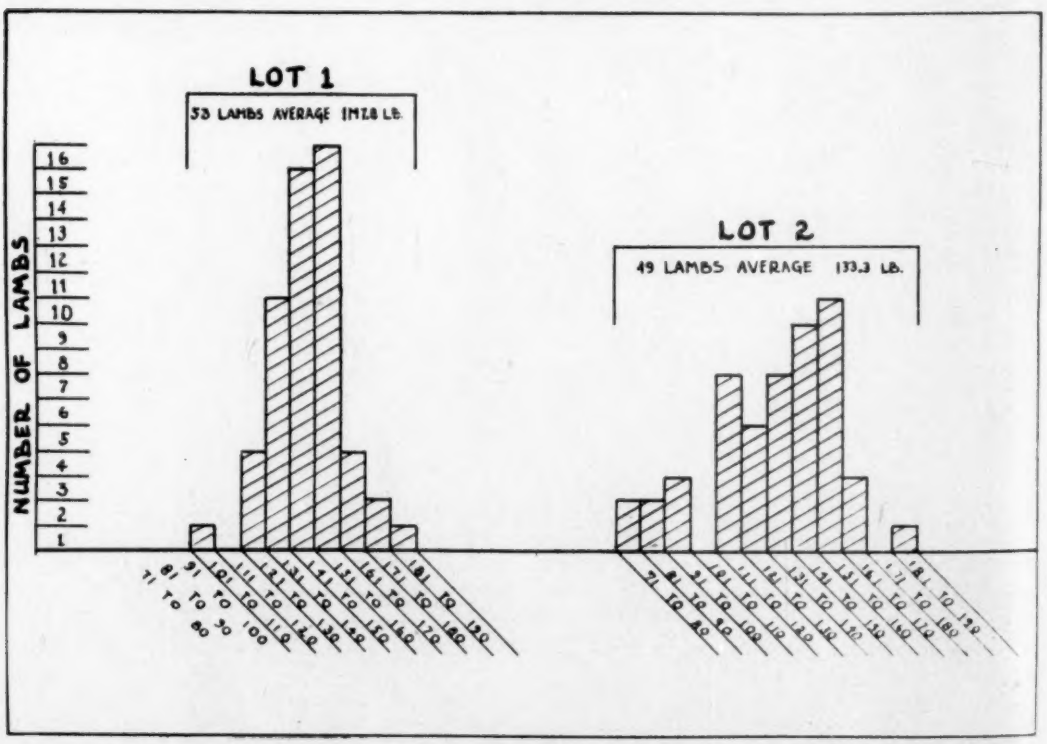

Fig. 2—Frequency histograms of weights of the two lots of lambs on Feb. 21, 1946, four months after treatment.

The weight differences between the 20-lamb samples of the two lots corresponded well with the weight differences for the entire lots, although the final difference between averages for the sample groups was less than for the entire lots. Figure 3 shows frequency histograms for the weights of the sample groups on Aug. 10, 1945, Jan. 8, 1946, and Feb. 21, 1946. No individual weights were obtained on October 22, just before treatment.

In addition to the evidence of fecal egg counts and weights as indicating the difference between the treated and untreated lots of sheep, further evidence exists in the results of the culling and wool grading of the entire lot of rams by the Department of Animal Industry, which was applied to all the lambs without reference to the division into two lots for the anthelmintic test. Out of the total of 108 rams, 52 were retained for the Station flock, and 56 were culled. On account of faulty records, there were 5 sheep of the 108 which could not be identified as to whether they were in the treated lot or the control lot. Of the 103 which were identified, 31 out of 54 culled were from the control lot, while 23 were from the treated. Of the culled group, 7 were in such poor condition that they were thrown out of the cull group as valueless. These 7 sheep all originated in the untreated lot.

The fleece weight averages showed only a slight difference, the average for the treated lot being 8.35 lb., while the average for the control lot was 8 lb. Fifteen fleeces were classified as "tender," of which nine originated from the untreated sheep.

After the close of the experimental period, 2 of the cull lambs were killed for comparative examination, 1 from the treated lot (A-2102) and 1 from the control lot (A-5080). When these 2 lambs were brought down from the summer range on August 10, A-5080 weighed 6 lb. more than A-2102. On Feb. 21, 1946, A-2102 weighed 135 lb., while A-5080 weighed 76 lb. At the beginning of the experiment, both had high fecal egg counts, 3,530 for A-2102, and 3,840 for A-5080. On Jan. 26, 1946, the egg count for A-2102 was 100, while for A-5080, it was 350. The lambs were killed on March 9. The worm counts were as follows:

A-2102

Abomasum	400 *O. circumcincta* and *O. trifurcata*.
	20 *T. colubriformis*.
Small Intestine	600 *T. colubriformis*.
	120 Nematodirus spp.
Colon	Few *Chabertia ovina*.
Fecal egg count	270 per gram.

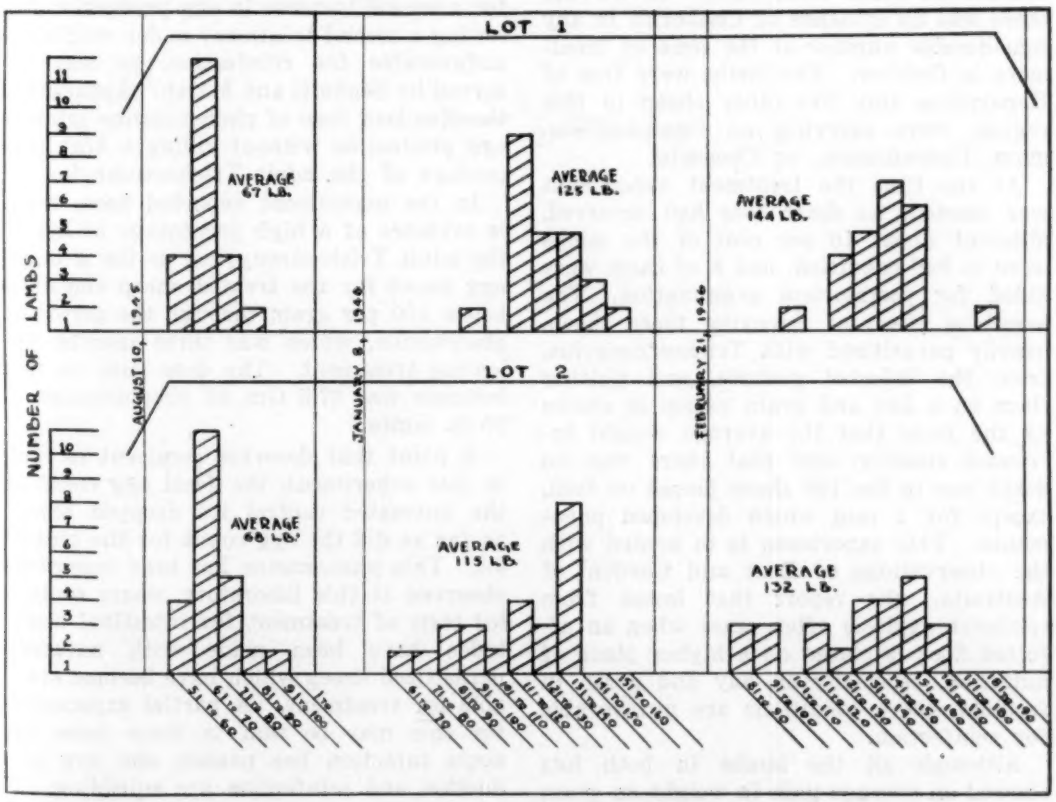

Fig. 3—Frequency histograms of weights of the 20-lamb samples of the two lots on Aug. 10, 1945, Jan. 8, 1946, and Feb. 21, 1946.

A-5080

Abomasum ...160 *O. circumcincta;* 4,500 *T. axei* and *T. colubriformis.*
Small intestine 1,500 *T. colubriformis* and *T. vitrinus.*
Colon .....Numerous *Chabertia ovina.*
Fecal egg count .....300 per gram.

There was a marked difference in the number of Trichostrongylus present in these 2 sheep, there being 6,000 counted in the control sheep, as compared with 620 in the treated sheep. The clinical difference may have been partly due to the Chabertia infection in the colon of A-5080, as the colon showed thickened walls caused by the Chabertia infection.

DISCUSSION

The experiment reported here appears to be worth recording for several reasons. In the first place, the sheep used were clinically parasitized with one or more species of Trichostrongylus in large numbers, to the practical exclusion of other intestinal nematodes. These sheep were carrying small numbers of Ostertagia and Nematodirus, and the January fecal egg counts showed Chabertia eggs in considerable number in some individuals. However, there was no evidence of Chabertia in any considerable number at the time of treatment in October. The lambs were free of Hemonchus and, like other sheep in this region, were carrying no Oesophagostomum, Bunostomum, or Cooperia.

At the time the treatment experiment was started, no death loss had occurred, although about 10 per cent of the lambs were in bad condition, and 3 of them were killed for postmortem examination. The beneficial effect of removing these sheep, heavily parasitized with Trichostrongylus, from the infected pasture, and putting them on a hay and grain ration is shown by the facts that the average weight increased steadily, and that there was no death loss in the 109 sheep placed on feed, except for 1 ram which developed pneumonia. This experience is in accord with the observations of Ross and Gordon[5] of Australia, who report that losses from trichostrongylosis often cease when an affected flock is placed on a higher plane of nutrition, especially on hay and grain in feedlots where conditions are unfavorable for reinfection.

Although all the lambs in both lots showed an average gain in weight on grain feed, it is also true that there was a marked additional increase in weight in the lot which was treated with phenothiazine. This difference in weight would have more than paid for the cost of treatment if this had been a bunch of feeder lambs. As this was a flock of purebred rams, the difference showed in both weight and quality, in that 31 of 54 lambs culled were from the untreated lot and, of these 54 culls, 7 were cut back as valueless, these 7 all being from the untreated lot.

Previous experience at this station had shown that phenothiazine in doses of 12.5 Gm. for lambs and 25 Gm. for adult sheep would produce an immediate reduction in fecal count of Trichostrongylus eggs to almost zero, but that in thirty to sixty days following treatment the egg count would have returned to a rather high figure, even where there was no opportunity for reinfection. This was the case with winter treatment of ewes reported by Seghetti and Marsh.[4] Gordon has shown that phenothiazine is ineffective against immature Trichostrongylus, but this could not account for a second increase in egg production following a second treatment under conditions unfavorable for reinfection, as was observed by Seghetti and Marsh.[4] Apparently, the standard dose of phenothiazine inhibits egg production without killing a high percentage of the adult Trichostrongylus.

In the experiment reported here, there is evidence of a high percentage of kill of the adult Trichostrongylus, as the average egg count for the treated sheep remained below 100 per gram through the period of observation, which was three months following treatment. The dose used in this instance was 37.5 Gm. of phenothiazine to 95-lb. lambs.

A point that deserves comment is that, in this experiment, the fecal egg count of the untreated control lot dropped almost as far as did the egg count for the treated lot. This phenomenon has been repeatedly observed at this laboratory where controlled tests of treatment for intestinal nematodes have been made with naturally parasitized sheep which have become available for treatment. A partial explanation for this may be that in these cases the acute infection has passed, and egg production and reinfection are subsiding. If this is a proper interpretation, it might account for the apparent good results of

certain treatments in practice, which cannot be substantiated in controlled experiments. It is also evident that the fecal egg count for Trichostrongylus is not necessarily correlated at all times with the pathologic effect on the host animal.

CONCLUSIONS

As a result of the experiment reported here, it has been concluded that phenothiazine in a dose of 37.5 Gm. for 95-lb. ram lambs was effective in controlling trichostrongylosis, under conditions unfavorable for reinfection; and that the increased weight and quality of the treated lambs more than paid the cost of treatment, even when applied after the acute infection period was passed and the sheep had been placed on grain feed.

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Streptomycin in the Treatment of Experimental Brucellosis of Guinea Pigs

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STREPTOMYCIN,* derived from *Actinomyces griseus*, was first announced in 1944 by Schatz, Bugie, and Waksman.[1] In general, it has proved useful in the treatment of certain gram-negative infections such as tularemia and some genito-urinary infections. Reiman, Elias, and Price[2] reported the recovery, after treatment, of 3 out of 5 typhoid fever patients. They call attention to the fact that different strains of *Eberthella typhosa* vary in their resistance to the product. Welch, Price, and Randall,[3] working with experimental *E. typhosa* infection in mice, reported that streptomycin at certain concentration levels when injected intraperitoneally into white mice increased, rather than decreased, the fatality rate. These results emphasize the need for proper dosage under controlled conditions. Feldman and Hinshaw,[4] using the antibiotic agent in the control of experimental tuberculosis in guinea pigs, concluded that "it is capable of exerting a striking suppressive effect on the pathogenic proclivities in guinea pigs" of the human type of *Mycobacterium tuberculosis*. Youmans and McCarter[5] obtained similarly encouraging results with experimental pulmonary tuberculosis in mice. Its effectiveness against the Brucella group has not been clearly evaluated. Reports on its use in the treatment of human brucellosis are meager and inconclusive. Jones and coworkers[6] found streptomycin to be effective in the prevention of *Brucella abortus* infection in chicken embryos. Santivanez[7] reported streptomycin to have a marked bactericidal action against *Br. abortus in vitro*. Live, Sperling, and Stubbs,[8] in a paper published during the course of the experiments to be reported here, concluded that, in the treatment of guinea pigs experimentally infected with a virulent strain of *Br. abortus*, with 5,000 units of streptomycin daily, divided into five doses, it had a definite bacteriostatic effect on the organism. In some of the animals, treatment was continued for twenty-seven days; in others, twenty days. Treatment of another group was initiated one week after infection, and continued twenty-four days. The administration of 20,000 units daily, divided into six doses, seemed to eliminate the infection in most of these animals.

METHODS

The present experiments were initiated in an attempt to determine the value of streptomycin as a preventive or curative agent in experimental brucellosis in guinea pigs. Guinea pigs were infected with varying numbers of *Br. abortus*. Streptomycin, administered in variable doses for varying periods, was started at variable intervals after infection. In one experiment, the drug was injected prior to infection. All guinea pigs weighed approximately 500 Gm. when infected. The infective organisms were injected subcutaneously. Streptomycin, suspended in sterile distilled water, was given intraperitoneally. All animals were killed five weeks after infection. Blood was collected for the agglutination test. Gross pathologic lesions were noted. The spleen of each animal was removed aseptically for culture on tryptose agar. Cultures were discarded only after incubation for two weeks. The value of blood-level determinations in the use of antibiotics was recognized. We planned to conduct such determinations, but the pressure of other work rendered this phase of the problem impossible. It was, therefore, decided to proceed without the benefit of the knowledge these determinations would give.

In general, the guinea pigs were divided into groups of 14, 4 of which were left as controls. There were a few deviations from this plan.

Presented before the Section on Research, Eighty-third Annual Meeting, American Veterinary Medical Association, Boston, Mass., Aug. 18-22, 1946.

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*The streptomycin was furnished by Merck and Co., Rahway, N.J.

The strain of *Br. abortus* used had been recovered from a virulent outbreak of bovine brucellosis. It was highly virulent at the time of use, and had been adapted to growth in the presence of air. All organisms injected were from 24-hour-old cultures. Sterile physiologic saline was used as a diluent.

Experiment 1.—All 14 guinea pigs were injected with 1,000,000 organisms. Ten were treated; 4 left as controls. The experiment was conducted to determine if streptomycin administered one hour prior to injection of the organisms would prevent development of the infection. Five animals each received 5,000 units of streptomycin one hour prior to infection. Each of the other 5 received 10,000 units one hour prior to infection. In none of the animals, treated or nontreated, was the infection prevented to any observable extent. All animals showed typical lesions and gave positive spleen cultures.

Experiment 2.—The guinea pigs in this experiment were divided into three groups of 7 pigs each — 5 animals in each group were treated, and 2 left as controls. All animals were infected with 1,000,000 organisms administered subcutaneously. In the first group, the 5 treated pigs were each given 5,000 units of streptomycin in one dose at the same time as infection. In the second group, treatment consisted of 5,000 units administered simultaneously to infection, and 5,000 units one hour later. Each animal in the third group received 1,000 units at the time of infection, and 1,000 units every succeeding hour, until each pig had received a total of 5,000 units. In each of these groups, the streptomycin so administered had no apparent effect on the course of the infection. All guinea pigs, treated and controls, showed the usual lesions of brucellosis, with high blood titers. *Br. abortus* was recovered from the spleen of each pig.

Experiment 3.—The guinea pigs in this experiment were divided into two groups of 14 each — 10 animals in each group were treated, and 4 left as controls. All animals were infected with 1,000,000 organisms. In the first group, treatment consisted of 2,000 units injected three hours after infection, followed by 2,000 units every morning and evening until each pig had received a total of 40,000 units. The second group was treated in a similar manner to the first group, except that treatment was started one week following infection. All animals at the time of slaughter were infected, as evidenced by high blood titers and positive spleen cultures. However, the spleens and lymph glands, particularly those from animals in which treatment was initiated three hours after infection, were noticeably smaller than in the controls. There appeared to be a definite bacteriostatic effect on the infection in the treated animals. It is possible that prolonged treatment might have produced better results in combating the infection.

Experiment 4.—This consisted of two groups of 14 guinea pigs each, 4 animals in each group being left as controls. All animals were infected with 1,000,000 organisms. Half of each group was treated with 5,000 units of streptomycin, followed by 2,000 units every two hours until each pig received a total of 19,000 units. The other 5 treated animals in each group received 10,000 units as an initial dose, and 5,000 units every two hours until each animal had received a total of 45,000 units. In the first group, treatment was started two hours after infection, while in the second group treatment was delayed until one week after infection. As in the previous experiment, there was no recovery from the infection nor was its development prevented. A noticeable bacteriostatic effect of the drug was observed in some of the treated animals. The lesions were less marked than in the controls. A few animals showed no observable lesions, but spleen cultures were positive. The cultures were in the form of scattered colonies rather than heavy growth, as in the controls. There appeared to be no difference in the degree of infection in those receiving 45,000 units over those receiving 19,000 units. All control animals showed typical lesions and positive cultures.

Experiment 5.—The animals in this group were divided into three groups, each consisting of 10 treated animals and 4 controls. All were infected with 50,000 organisms. In the first group, treatment was initiated two hours after infection. Five of the animals received 5,000 units as an initial dose. This was followed by succeeding doses of 2,000 units every two hours until each animal received a total of 19,000 units. The other 5 received an initial dose of 10,000 units, followed by 5,000 units every two hours until each animal received a total of 45,000 units. In the second group, treatment was initiated one week after infection. Five of the animals received an initial dose of 5,000 units, followed by 2,000 units every two hours until each animal received a total of 19,000 units. The other 5 received 10,000 units at the first dose, and 5,000 units each succeeding two hours until each pig received a total of 45,000 units. In the third group, treatment was started two weeks after infection. Five of the treated animals received a first dose of 10,000 units and 5,000 units every two hours thereafter until each pig received a total of 45,000 units. The other 5 received 10,000 units at the first dose, and 10,000 units every two hours until each received a total of 80,000 units.

As in the previous experiments, treatment with streptomycin failed to overcome the infection in any of the treated animals. Nevertheless, there was a very noticeable bacteriostatic effect. Practically all the treated animals showed less extensive lesions than the controls and, in several instances, lower agglutination titers. Some animals showed only scattered colonies on spleen culture, in contrast to heavy growth on the cultures made from the spleens of control animals. In the first group, in which treatment was started two hours after infection, 1 of the animals receiving 5,000 units as an initial treatment

failed to show any observable lesions *post mortem*. In the same group, 3 of the 5 receiving 10,000 units on initial treatment showed no observable postmortem lesions. In the second group, all those receiving 5,000 units as a first dose showed some lesions, while all 5 receiving the initial inoculation of 10,000 units gave no gross evidence of infection. All animals in the third group, in which treatment was started two weeks after infection, showed some lesions, but to a lesser degree than the controls. All controls showed good lesions, high agglutination titers, and positive cultures. The 5 animals in the third group treated with 80,000 units showed marked depression following the fourth 10,000-unit injection. One animal was markedly prostrated but recovered. All animals started to revive about two hours later and were merely drowsy when treatments ceased. They were apparently normal the next morning. No other animals in this experiment showed any apparent toxic effect as a result of the streptomycin injections.

Experiment 6.—The animals in this experiment were divided into three groups, each consisting of 10 treated pigs, and 4 controls. All were infected with 10,000 organisms. In the first group, treatment was initiated two hours after infection. Five of the 10 treated animals received 10,000 units on initial inoculation, and 2,000 units every succeeding two hours until each pig received a total of 24,000 units. The other 5 received 10,000 units as a first dose, followed every two hours by 5,000 units until each animal had received a total of 45,000 units. In the second group, treatment was started one week after infection. Half of the 10 treated animals in this group received an initial dose of 10,000 units and 2,000 every two hours until each pig received 24,000 units. The other 5 received 10,000 units, followed every two hours with 5,000 units until each pig received a total of 45,000 units. In the third group, treatment was started two weeks after infection. Half of the group received 10,000 units, followed by 5,000 units every two hours until each pig received a total of 45,000 units. The other 5 pigs received 10,000 units every two hours until each pig received a total of 80,000 units.

Of the 5 animals in the first group that received a total of 24,000 units, 2 showed no observable lesions on autopsy. Agglutination tests were lower than in the other 3. The spleen of 1 gave moderately heavy growth, while the other yielded only scattered colonies. The other 5 animals in this group (receiving a total of 45,000 units) yielded 2 with no observable lesions. One gave a negative agglutination test and negative spleen cultures; the other yielded scattered colonies. Three of the 4 controls in this group showed no lesions and were negative to the agglutination test and spleen cultures. The other control died three weeks after infection. Spleen cultures were positive. No agglutination test was performed. The lack of infection in 3 of the controls is difficult to explain. It is possible, but unlikely, that we failed to inoculate them with the organisms, or that they were animals with a high degree of natural immunity. The infective dose of 10,000 organisms is above the minimum infective dose for guinea pigs.

In the second group, in which treatment was started one week after infection, 1 of the 5 receiving a total of 24,000 units, and 1 receiving a total of 45,000 units, showed no observable lesions. The spleen of 1 yielded scattered colonies; the other, good growth. All 4 controls were definitely positive. In general, most of the treated animals developed less extensive lesions than the controls.

There was no observable difference in the extent of the brucellosis infection in the third group, in which treatment was started two weeks after infection. The controls were all positive. Controls and treated animals all showed typical brucellosis lesions. All were positive to agglutination test and cultures.

DISCUSSION AND CONCLUSIONS

Streptomycin, as used in these experiments, failed to overcome or prevent experimental brucellosis. It did have a noticeable bacteriostatic effect on the course of the infection in many of the animals used, particularly when treatment was prolonged. It is very probable that had we injected the drug in a manner to cause slower absorption, such as intramuscularly or subcutaneously and, had the treatment been continued over longer periods, the results might have been more favorable. Kornegay and associates[8] have found the intramuscular and subcutaneous routes to be most favorable for prolonged maintenance of high blood levels. The work of Live and his coworkers[5] have demonstrated the effectiveness of the drug when administered over longer periods.

DISCUSSION†

Q.—I would like to know the price at present of streptomycin, and what it would cost to treat a guinea pig.

DR. GILMAN.—That is one thing we did not have to worry about. The streptomycin was furnished by Dr. Green of Merck and Company. I believe I saw in the paper that streptomycin sold for $20.00 a gram. That was at the time of the black market scandals. I do not know what it costs now. I do not believe anyone does. I understand it is under the distribution of Dr. Kiefer now, and that it is distributed free. It is all being allocated, as Dr. Karlson said yesterday.

DR. GWATKIN.—How was it injected?

DR. GILMAN.—Subcutaneously.

DR. GWATKIN.—I wonder how much differ-

†After the presentation of the paper, the following discussion was conducted.

ence it would make if they were injected by eye. I remember years ago that we protected guinea pigs by giving them the serum at the same time or a few hours later but, if they were injected, we couldn't protect them. I do not know about the size of the dose. As I remember, they were about the critical doses. I wonder if that would have any bearing, purely from the experimental angle.

DR. GILMAN.—Well, I do not know. I really think, though—and I believe Dr. LeGrow will agree with me—that had we injected these pigs morning and night over a longer period of time, we would have had excellent results, even in the heavy infections with a million organisms. We did not keep at it long enough. It is rather surprising what the drug will do. But it must be remembered that streptomycin is expensive, and also that tremendous doses would be necessary for cattle. Furthermore, as everyone understands, streptomycin does not seem to work if the infection is well established, that is, for over two weeks. Those are the results of everyone who has worked with the product so far.

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The Chemotherapy of Cecal Coccidiosis

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THE USE of certain sulfonamides has shown promise as a means of preventing and treating cecal coccidiosis in poultry. Herein is reported a series of experiments designed to evaluate some of the sulfonamides and other newer chemicals.

The investigations of Levine[1, 2, 3] in which he tested sulfanilamide, sulfapyridine, and sulfaguanidine were the first extensive reports on the use of sulfonamides. Farr and Allen[4] also tested sulfaguanidine against *Eimeria tenella* infection. Horton-Smith and Taylor[5, 6] showed that sulfamethazine had inhibitory effects on cecal coccidiosis when administered late in the development of the infection. Hawkins[7] reported favorably on the use of sulfamethazine for cecal coccidiosis. Swales[5, 9, 10] showed that sulfamerazine and sulfamethazine had definite therapeutic value for cecal coccidiosis. Farr and Wehr[11, 12] reported on sulfamerazine and sulfaguanidine as agents for cecal coccidiosis. They observed gross lesions, which appeared to be due to the effects of the drug, in a number of the sulfamerazine experiments. Seeger[13] showed that sulfaguanidine gave a high protection against losses in chicks receiving large doses of *E. tenella* oöcysts and that chicks surviving the initial inoculation, both treated and untreated, were immune to second inoculation. In a preliminary report, Thorp *et al.*[14] noted similar beneficial results from the use of sulfamerazine as reported by Swales, Hawkins, and others.

The sulfonamide, 2-sulfanilamido-4-methylpyrimidine (sulfamerazine), was used in these experiments and, throughout the report, it is referred to as sulfamerazine. This is the insoluble sulfamerazine powder employed in the experimental mash. The soluble sodium salt (Sulfowl) which was used in the drinking water is referred to as sodium sulfamerazine. This report contains further data on the use of these sulfonamides prophylactically and therapeutically for cecal coccidiosis after symptoms develop.

EXPERIMENTAL PROCEDURE

The Barred Plymouth Rock chickens used in the experiments were coccidiosis-free fowl obtained from the Department of Poultry Husbandry, Pennsylvania State College. In each experiment, 100, 4-week-old chickens were divided into five pens of 20 each. These chicks were placed in clean batteries which were free from any infectious material. All of the chickens in each experiment were from the same hatch and were distributed evenly throughout the pens on the basis of live weight at 4 weeks of age. Ten experiments were set up and carried out at different times under identical conditions. With the exception of two experiments, the chickens were weighed every other day except Sundays. Autopsies were held on all surviving chickens, and they were examined for gross lesions of cecal coccidiosis and for any lesions suggesting toxicity. Tissues for microscopic study were saved from each chicken which showed gross lesions in the liver, spleen, and kidney, and from all surviving chickens of four experiments. All chickens which were inoculated orally were given a suspension of infective, sporulated *E. tenella* oöcysts. Infectivity, as determined by previous trials with a small number of susceptible chickens as well as the number of sporulated oöcysts per inoculation, was the basis used in determining the amount of inoculum. The average number of sporulated oöcysts given was from 75,000 to 100,000, but the infectivity of the oöcysts was a better criterion upon which to base the dosage of inoculum.

Authorized for publication Aug. 5, 1946, as paper No. 1336 in the journal series of the Pennsylvania Agricultural Experiment Station from the Animal Pathology Laboratory of the Department of Animal Husbandry.

Presented before the Poultry Section of the Eighty-third Annual Meeting, American Veterinary Medical Association, Boston, Mass., Aug. 18-22, 1946.

Professor (Thorp): assistant professor (Gordeuk); assistant (Glantz); and assistant (Learned); Pennsylvania State College Agricultural Experiment Station, State College.

The authors express their appreciation to the Department of Poultry Husbandry, Pennsylvania State College, for its coöperation in furnishing the 4-week-old chickens and necessary feed; to Mr. T. C. Simmons, of the Department of Agricultural and Biological Chemistry, for the blood determinations; and to the Medical Research Division of Sharp and Dohme, Inc., for furnishing the sulfamerazine and research grant in partial support of these studies.

A brief outline of the experiments reported will serve to describe the various tests. They are as follows:

Experiment 1.—Pen 1.—At symptoms of coccidiosis, sodium sulfamerazine was administered for five days in a concentration of 2.0 Gm. per liter of drinking water.

Pen. 2.—At symptoms of coccidiosis, a 1.0 per cent sulfamerazine mash was administered for five days.

Pen 3.—A 1.0 per cent sulfamerazine mash was administered for five days, but no infective oöcysts were given.

Pen 4.—Untreated, uninoculated control.

Pen 5.—Untreated, but inoculated, control.

Experiment 2.—Pen 1.—A 0.25 per cent sulfamerazine mash was administered as a prophylactic for five days beginning on the day of inoculation.

Pen 2.—Sodium sulfamerazine was administered in a concentration of 1.0 Gm. per liter of drinking water as a prophylactic for five days beginning on the day of inoculation.

Pen 3.—A 0.25 per cent sulfamerazine mash was administered for five days, but no infective oöcysts were given.

Pen 4.—Untreated, uninoculated control.

Pen 5.—Untreated, inoculated control.

In this experiment, all the survivors were given infective oöcysts two weeks following recovery from the first infection.

Experiment 3.—Pen 1.—At symptoms of coccidiosis, sodium sulfamerazine was administered for five days in a concentration of 2.0 Gm. per liter of drinking water.

Pen 2.—Sodium sulfamerazine was administered in a concentration of 2.0 Gm. per liter of drinking water for five days, but no infective coccidia were given.

Pen 3.—A 1.0 per cent sulfamerazine mash was administered for five days, but no infective oöcysts were given.

Pen 4.—A 0.25 per cent sulfamerazine mash was administered for five days, but no infective oöcysts were given.

Pen 5.—Untreated, inoculated controls.

Pens 2, 3, and 4 were used for toxicity studies and determination of sulfamerazine concentration in the blood.

Experiment 4.—Pen 1.—At symptoms of coccidiosis, sodium sulfamerazine was administered for four weeks at the rate of 2.0 Gm. per liter of drinking water.

Pen 2.—At symptoms of coccidiosis, a 0.25 per cent sulfamerazine mash was administered and continued for four weeks.

Pen 3.—A 0.25 per cent sulfamerazine mash was administered for four weeks, but no infective oöcysts were given.

Pen 4.—Untreated, uninoculated control.

Pen 5.—Untreated, inoculated control.

Experiment 5.—At symptoms of coccidiosis, sodium sulfamerazine in a concentration of 2.0 Gm. per liter of drinking water was administered to pens 1, 2, 3, and 4 for five days. Pen 5 was not treated but was inoculated with infective oöcysts at the same time as all other pens. Beginning one week following the five days of treatment, 10 per cent of the survivors of each pen were removed for autopsy and tissue studies.

Experiment 6.—At symptoms of coccidiosis, a 0.5 per cent sulfamerazine mash was administered for five days to pens 1, 2, 3, and 4. The birds in pen 5 were not treated but were inoculated with infective oöcysts at the same time as all other pens. Beginning one week following the five days of treatment, 10 per cent of the survivors of each pen were removed for autopsy and tissue studies.

Experiment 1 was repeated once, and two other groups of chickens, totaling 100 each, were used as checks for several of the experiments. A number of field outbreaks were treated with procedures similar to those used in the artificially infected groups. The methods used for determining the blood concentration of free sulfamerazine were those commonly employed in laboratories making such chemical determinations.

TABLE 1—The Results of Sulfamerazine Administration on the Course of Eimeria Tenella Infection in Experiment 1

Pen no.	No. chickens	Dosage	Av. gain Gm.	Mortality %	Survivors with gross lesions of *E. tenella* None	Survivors with gross lesions of *E. tenella* Present	Gross lesions spleen and liver None	Gross lesions spleen and liver Present
1	20	0.2% in water	80.2	20	1	15	13	3
2	20	1.0% in mash	62.0	15	0	17	11	6
3	20	1.0% in mash	101.0	0*	20	0	16	4
4	20	None	153.8	0*	20	0	15	5
5	20	None	76.1	45	0	11	4	7

*No infectious oöcysts given.

DISCUSSION

The results obtained in these experiments were, for the most part, in agreement with those reported elsewhere. This report deals entirely with the use of sulfamerazine for cecal coccidiosis.

The information obtained from the first experiment is shown in table 1. These

tests were conducted to determine any significant differences between the administration of a 0.2 per cent sodium sulfamerazine drinking water and a 1.0 per cent sulfamerazine mash given at the onset of symptoms of coccidiosis. There was a 5 per cent difference in mortality between the two treatments and a significant difference in average gains of the two groups. A marked difference was noted in the average gains between pen 2, given infective oöcysts and 1.0 per cent sulfamerazine mash, and pen 3, which received the 1.0 per cent sulfamerazine but no infective oöcysts. On this basis, one would assume that at least a portion of the difference in weight gain was due to the coccidiosis. Pen 4, which received no infective oöcysts and no treatment, made the best gains, indicating some interference with growth when the drug was given at the 1.0 per cent level in the mash. The average gains for the untreated, infected group (pen 5) was next to the lowest, but after a 45 per cent mortality, the remaining chickens averaged somewhat higher in weight than the recovered chickens. The gross lesions observed in the spleen and liver at autopsy of the surviving chickens did not follow any consistent pattern. Taking all experiments into consideration, there were as many lesions in the untreated control group as in the treated control group, although the percentage in some experiments was slightly higher for the untreated controls.

The second experiment was carried out to determine whether the prophylactic value of sulfamerazine was similar to that reported for sulfaguanidine,[2] to determine the effect of a 0.25 per cent sulfamerazine mash and a 0.1 per cent sodium sulfamerazine drinking water upon the average gains, and to show the prophylactic value of these dosages. The results of this experiment are shown in table 2. It will be noted that the 0.25 per cent sulfamerazine mash (pen 3) apparently did not have any deleterious effect upon the average gains during treatment. The chickens receiving infective oöcysts but no treatment (pen 5) made the lowest gains. The chickens receiving no infective oöcysts (pens 3 and 4) made the highest average gains in the first part of the experiment and the lowest average gains upon reinfection. The highest average gain for the second part of the experiment was made by pen 1, receiving 0.25 per cent sulfamerazine mash. In both the 0.25 per cent sulfamerazine mash (pen 1) and 0.1 per cent sodium sulfamerazine drinking water (pen 2) groups, there was a 10 per cent mortality as against a 50 per cent mortality for the untreated control group (pen 5). When the five pens were reinfected, pens 3 and 4, which had not been previously infected with coccidiosis, sustained a 40 and 45

TABLE 2—The Results of Sulfamerazine Administration on the Course of Infection with Eimeria Tenella When Used as a Prophylactic in Experiment 2

Pen no.	No. chickens	Dosage	Av. gain Gm.	Mortality %	Survivors with gross lesions of *E. tenella* None	Survivors with gross lesions of *E. tenella* Present	Gross lesions spleen and liver None	Gross lesions spleen and liver Present
1	20	0.25% in mash	111.8	10				
2	20	0.1% in water	96.1	10				
3	20	0.25% in mash	145.5	0*				
4	20	None	139.0	0*				
5	20	None	50.5	50				
Results upon Reinfection of All Five Pens Two Weeks Following Recovery from First Infection								
1	18		211.1	0	15	3	16	2
2	18		184.5	0	6	12	17	1
3	20		186.3	40*	2	10	9	3
4	20		178.9	45*	0	11	9	2
5	10		188.5	0	0	10	6	4

*No infectious oöcysts on first test.

per cent mortality. It should be noted that these chickens were 7 weeks old and received the same dosage of oöcysts as the other groups at 4 weeks of age. These chickens infected in the first part of the experiment did not sustain any mortality upon reinfection, indicating that immunity was produced by the first infection. Consumption of 0.25 per cent sulfamerazine mash and 0.1 per cent sodium sulfamerazine drinking water apparently did not hinder the development of immunity in this experiment.

The third experiment, reported in table 3, was developed for the purpose of comparing blood concentrations of sulfamerazine when 0.2 per cent sodium sulfamerazine drinking water, 1.0 per cent sulfamerazine mash, and 0.25 per cent sulfamerazine mash were administered for a five-day period. The experiment also served as a recheck of experiment 1, in which a 0.2 per cent drinking water solution was given at the first symptoms of cecal coccidiosis. There was a marked difference in the average gains of the treated, infected group (pen 1) and the untreated, infected group (pen 5) and a 30 per cent difference in mortality. The concentration of free sulfamerazine in the blood of chickens from pens 2, 3, and 4, treated for this purpose, is shown in table 4. The 1.0 per cent mash (pen 3) produced extremely high blood levels of free sulfamerazine. Drinking water containing 0.2 per cent of sodium sulfamerazine (pen 2) produced higher blood concentrations than did the 0.25 per cent sulfamerazine mash (pen 4). No toxic symptoms or blood changes were observed in any of the groups.

In the first three experiments, some evidence indicated that the administration of sulfamerazine in various dosages affected the average gains. The fourth experiment was designed to determine any toxic effect from prolonged administration of sulfamerazine at a concentration of 0.2 per cent in the drinking water and 0.25 per cent in the mash. These dosages were given for four weeks beginning at the onset of symptoms in two infected groups. The birds of pens 3 and 4, receiving no infective oöcysts, made the highest average gains, although those of pen 2 were given the 0.25 per cent sulfamerazine mash for the four weeks. There was no increase in the number of lesions in the spleen and liver compared to those of chickens on

TABLE 3—The Results of Sulfamerazine Administration on the Course of Eimeria Tenella Infection in Experiment 3

Pen no.	No. chickens	Dosage	Av. gain Gm.	Mortality %	Survivors with gross lesions of *E. tenella* None	Survivors with gross lesions of *E. tenella* Present	Gross lesions spleen and liver None	Gross lesions spleen and liver Present
1	20	0.2% in water	75.5	15	2	15	16	1
2	20	0.2% in water	54.5	0*	20	0	17	3
3	20	1.0% in mash	44.2	0*	20	0	19	1
4	20	0.25% in mash	43.8	0*	20	0	16	4
5	20	None	29.4	45	0	11	8	3

*No infectious oöcysts given, but blood determinations were made for concentration of free sulfamerazine—*see* table 4.

TABLE 4— The Concentrations of Free Sulfamerazine in the Blood of Chickens from Pens 2, 3, and 4 of Experiment 3

Wing band no.	1st day Mg./100 cc.	2nd day Mg./100 cc.	3rd day Mg./100 cc.	4th day Mg./100 cc.	5th day Mg./100 cc.
Pen 2					
151	12.50	12.22	14.63	9.14	15.61
158	11.30	12.05	16.31	8.94	14.40
139	15.59	16.06	14.99	7.60	16.08
166	11.57	16.38	16.03	15.01	17.29
Pen 3					
178	21.00	33.48	24.25	24.40	24.70
179	20.80	28.47	25.80	25.80	28.80
184	27.20	36.50	29.05	29.05	26.00
199	24.67	32.38	32.38	27.10	31.70
Pen 4					
205	10.12	7.48	8.62	7.74	8.81
213	10.00	7.46	8.41	5.77	8.42
214	10.91	10.09	9.43	5.49	6.98
216	9.92	8.10	7.10	8.31	6.03

other experiments treated for a shorter period.

The fifth and sixth experiments were undertaken to determine the extent of any toxicity which might appear during a reasonable period following treatment and recovery. The results of these tests are shown in table 6.

In experiment 5, a 0.2 per cent sodium sulfamerazine, drinking water solution was administered for five days beginning at the onset of symptoms of cecal coccidiosis and, in experiment 6, a 0.5 per cent sulfamerazine mash was used as the five-day treatment. In both experiments, following treatment and recovery for ten weeks, 10 per cent of the survivors were removed each week for autopsy, and tissues were saved for microscopic study. These two experiments were no more consistent than the other experiments regarding the small number of lesions found in the spleen and liver. A comparison of the average gains of the treated chickens in experiments 5 and 6, and the untreated inoculated controls for the concluding ten-week period, is shown in chart 1.

On the basis of the mortality in the various groups, the 0.5 per cent sulfamerazine mash appears to be a very satisfactory treatment. It should be remembered that the chickens used in these

TABLE 5—The Effect of Sulfamerazine Administration on Eimeria Tenella and Weight Gains When Given for Four Consecutive Weeks in Experiment 4

Pen no.	No. chickens	Dosage	Av. gain Gm.	Mortality %	Survivors with gross lesions of *E. tenella* None	Present	Gross lesions spleen and liver None	Present
1	20	0.2% in water	142.0	15	12	5	15	2
2	20	0.25% in mash	194.5	25	14	1	12	3
3	20	0.25% in mash	231.1	0*	20	0	17	3
4	20	None	243.3	0*	20	0	18	2
5	20	None	172.8	75	0	5	3	2

*No infectious oöcysts given.

TABLE 6—The Effect of Sulfamerazine on the Course of Eimeria Tenella Infection in Experiments 5 and 6

Pen no.	No. chickens	Dosage	Av. gain Gm.	Mortality %	Survivors with gross lesions of *E. tenella* None	Present	Gross lesions spleen and liver None	Present
			Experiment 5					
1	20	0.2% in water	83.3	15	3	14	17	0
2	20	0.2% in water	58.6	10	1	17	18	0
3	20	0.2% in water	66.9	15	7	10	17	0
4	20	0.2% in water	86.0	5	8	11	19	0
5	20	None	74.9	45	0	11	9	2
			Experiment 6					
1	20	0.5% in mash	185.0	0	12	8	20	0
2	20	0.5% in mash	152.1	0	6	14	19	1
3	20	0.5% in mash	147.2	0	15	5	20	0
4	20	0.5% in mash	162.2	0	10	10	17	3
5	20	None	105.8	60	0	8	6	2

*The gains shown in this table are for the first two weeks of the experiment; average weights for the ten-week period following are shown in chart 1.

experiments were given a very high dosage of infective oöcysts as compared to the probable intake of such oöcysts under field conditions. This was borne out by the satisfactory results obtained when five flocks totaling 3,200 chickens were treated with either a 0.2 per cent drinking water solution or a 0.25 per cent mash. In these flocks, diagnosis was made by autopsy and microscopic examination of cecal and intestinal contents. None of the five flocks showed coccidia other than *E. tenella*.

Extension of the experiments described in a preliminary report,[14] in which experiments using sodium sulfamerazine in combination with a small amount of sulfathalidine were given, did not indicate any advantage of this mixture over sodium sulfamerazine in the drinking water or sulfamerazine in the mash. On the basis of this experience, no advantage could be seen when the sulfathalidine was added to the sulfamerazine.

Upon repeating experiment 1, similar results were obtained. It was necessary to repeat experiments 1 and 5 because of the variation in oöcyst infectivity. In this type of experiment, all oöcysts should be tested on 12 or 15 chickens just before inoculating large groups regardless of their disease-producing qualities in previous experiments.

A study of the tissues saved from those experiments where histologic sections were made from all the chickens, and of those from other experiments providing tissues showing gross lesions, revealed some constancy in the microscopic pathology. The lesions in the spleen appeared as infarctions. In practically all instances, there was a hemorrhagic area surrounding the necrotic tissue. This was particularly true in the lesions observed in the spleen. The lesions in the spleen were consistently of longer duration than those in the liver. A study of tissues from future experiments may reveal more information on the character of the liver lesions; however, the lesions in the spleen can be described as small, hemorrhagic infarcts. In all lesions in the spleen, considerable organization of the central portion of the infarct

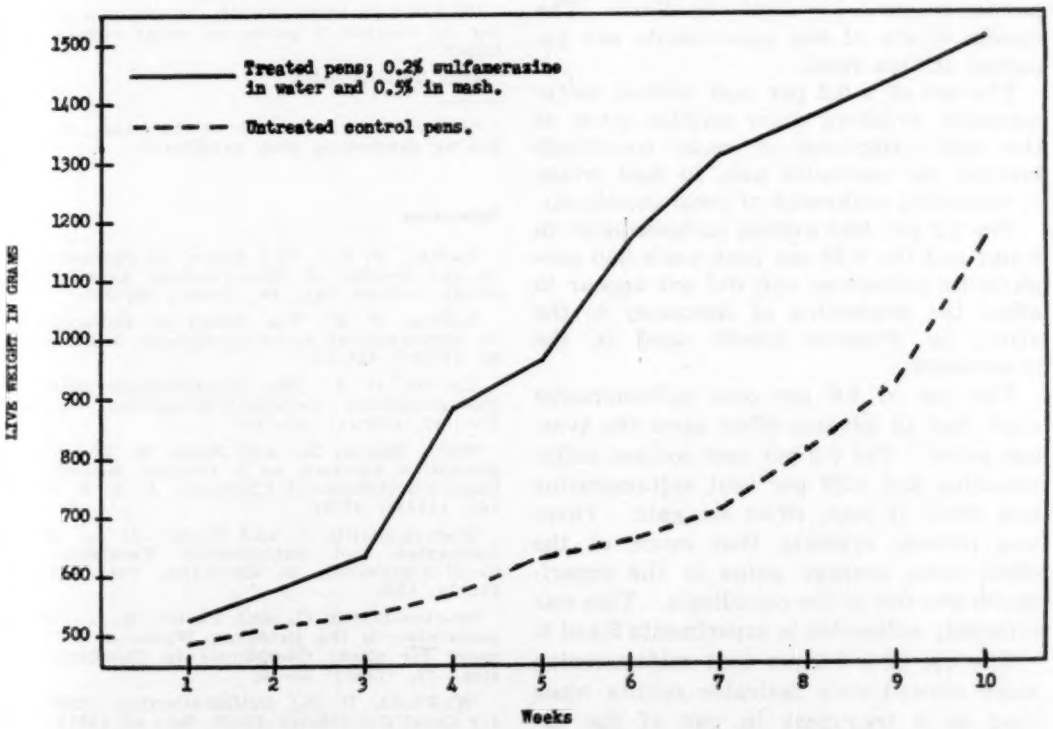

Chart 1—The average gains of the treated pens and the untreated controls for the ten-week period following infection with Eimeria tenella in experiments 5 and 6.

had taken place and was manifested by extensive proliferation of fibroblasts and some scar tissue. This was not the case in the liver. The liver lesions did not appear to be simple infarcts but rather appeared to be the result of necrosis of the parenchymatous cells followed by an infiltration of mononuclear cells and, to a lesser degree, polymorphonuclear leucocytes. These lesions consistently were smaller than those of the spleen. Further study of the tissues from the chickens showing gross lesions is being made. In only 1 per cent of the cases where sections were saved for microscopic examination without evidence of gross lesions was any pathology observed and then only in the liver tissue. No significant changes were observed in the kidney tissue from chickens on any of the experiments.

SUMMARY

A series of experiments using 1,000, 4-week-old chickens were carried out to determine the efficacy and toxicity of sodium sulfamerazine in water and sulfamerazine powder in mash for cecal coccidiosis. The details of six of the experiments are reported at this time.

The use of a 0.2 per cent sodium sulfamerazine, drinking water solution given at the first symptoms of cecal coccidiosis lowered the mortality and, in field trials, it controlled outbreaks of cecal coccidiosis.

The 0.2 per cent sodium sulfamerazine in water and the 0.25 per cent mash had prophylactic properties and did not appear to affect the production of immunity to the strain of *Eimeria tenella* used in the experiments.

The use of 1.0 per cent sulfamerazine mash had an adverse effect upon the average gains. The 0.2 per cent sodium sulfamerazine and 0.25 per cent sulfamerazine had little, if any, effect on gain. There was definite evidence that much of the effect upon average gains in the experiments was due to the coccidiosis. This was especially noticeable in experiments 5 and 6.

The use of a 0.5 per cent sulfamerazine mash showed very desirable results when used as a treatment in one of the experiments. This concentration in the mash will be repeated in future trials.

There was no consistent pattern regarding the lesions observed in the spleen and liver as related to dosage with sulfamerazine on infection with cecal coccidiosis. The gross lesions occurred in the spleen about three times as often as in the liver. Microscopic examination of the tissues did not substantiate this ratio, since microscopic lesions were about equally distributed between the spleen and liver. Since tissue pathology was encountered among both the untreated and treated chickens, the spleen and liver lesions could not be attributed to sulfonamide therapy at this time.

DISCUSSION*

CHAIRMAN DICKINSON.—You indicated, I think, in your first chart that some of the untreated group also showed those lesions.

DR. THORP.—Yes, they did. There wasn't any particular difference between the number of lesions in that group and in the others. I think it will have to be followed up again as far as the lesion is concerned. That was the reason for experiments 5 and 6 where we took out 10 per cent each week to see what the outcome was.

CHAIRMAN DICKINSON.—Would you be willing to venture a guess on what caused that lesion?

DR. THORP.—No.

*After the presentation of the paper, the following discussion was conducted.

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Studies in Pullorum Disease. XII. Antigenic Differences in Strains of Salmonella Pullorum

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YOUNIE,[1] in 1941, reported on difficulties encountered in controlling chick losses during the three previous years. During 1939, a few outbreaks of pullorum disease occurred in chicks from negative flocks which had been raised on premises free from possible infection. The next year, there were a large number of such outbreaks, and the majority occurred in one breed. A retest revealed only 11 reactors in some 10,000 birds. This led to Younie's observation that there was an antigenic difference in the strains responsible for the trouble. Both he[2] and Byrne[3] considered the possibility of quantitative, rather than qualitative, differences but drew no conclusions in this regard. Glover and Connell[4] found that strains of *Salmonella pullorum* examined by them fell into at least two groups, normal and variant.

EXPERIMENTAL METHODS

An experimental study was commenced by the Division of Animal Pathology in June, 1944. From a pullorum-free source, 408 New Hampshire chicks were obtained. Sixty were segregated for later contact exposure and to serve as evidence of the health of the others until that time. Ten died during transit or shortly afterwards and showed no evidence of pullorum infection. The remaining 338 were infected orally with 0.25 cc. of a suspension of heart, liver, and lungs of infected chicks. This material was obtained by injecting chicks with variant strains, removing, culturing, and freezing the organs, and using those that yielded pure cultures of the variant organism. The organs were ground with sand, and the supernatant of a 14 per cent suspension was employed. Fifty-nine died as a result of this exposure (17.4%), and *S. pullorum* was recovered from 34 (57.6 %). This flock was used as the basis of our preliminary work.

In the work reported in this paper, the tube agglutination test was conducted in the usual manner in dilutions of 1 : 25, 1 : 50, and 1 : 100, or serial dilutions of from 1 : 10 to 1 : 320 and higher. In some cases, only the test dilution of 1 : 50 was employed. Regular antigen refers to one prepared by the standard method from strains 17, 19, and 20. Variant antigen was prepared from Younie strains 6 and 296 or, in some cases, the latter alone. Tests were incubated for forty-eight hours at first but, later in the work, variant antigen was read at twenty-four hours. For the whole blood test, a crystal-violet antigen prepared from BAI strains 4, 10, and 11 grown on K medium was used for regular antigen and Younie strains 6 and 296, or the latter alone, for variant antigen. A great many other antigens were employed in comparative tests but, to avoid confusion, these results will not be introduced. Equal quantities of blood and antigen were used in the whole blood test, the loop and dropper each delivering about 0.04 cc. Readings at first were made at two minutes, then shortened to one minute, and are now made at thirty seconds.

Chicks were cultured on brilliant green agar plates (1 : 30,000) and blood agar or beef infusion agar. The pooled heart, liver, and lung were seeded on these plates. Broth cultures were made from the organs of adult birds, incubated twenty-four hours, and plated on brilliant green agar. Cultures were identified by seeding in lactose, galactose, maltose, glucose, mannitol, sucrose, salicin, and dulcitol broth. No differences were observed between regular and variant strains by ordinary bacteriologic methods, nor did a bacteriophage show any significant difference between ten strains which were serologically regular and six variants.[5]

RESULTS

Differences in Response of Regular and Variant Antigen.—Differences between regular and variant antigens were striking while the birds were young.[6, 7] The whole blood test on 294 chicks tested five to six weeks after exposure showed 191 reacted positively with variant antigen while only 20 reacted positively with regular. Grouping positive and suspicious reactions, 185 were not detected by regular antigen (89 %). The next test was made with both tube and whole blood tests 80 to 102 days after exposure, at which time the regular antigen failed to detect 131 (68%)

Presented before the Section on Poultry, Eighty-third Annual Meeting, American Veterinary Medical Association, Boston, Mass., Aug. 18-22, 1946.

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of the positives. Recovery of the organism from the ovaries and organs of many chicks that died, or were killed for examination during the experiment, afforded proof of the specificity of the variant strain reactions. In 815 tests of known negative birds, only 2 would have been removed (0.24%). An interesting observation was that, among the miscellaneous samples tested, 63 chicks injected with regular strains showed a preponderance of variant agglutinins six days after injection, but two months later, after further injections, the predominating agglutinins in most instances were of the regular type.

Failure of regular strains to detect infected birds was not of practical import in these early tests but, when the flock was divided into positive and negative groups at 5 1/2 months of age, the regular antigen, by both tube and whole blood methods, failed to detect 49 per cent of the reactors. On the next test, about one month later, 10 reactors were removed from the negative group by the variant antigens, and *S. pullorum* was recovered from 7. Five of the 10 were negative with regular antigen in both tube and whole blood tests, and 3 of these birds yielded *S. pullorum* on culture.

On the next test, 3 reactors were removed from the negative group on the basis of the variant antigens. Regular antigen had failed to detect the only 1 from which *S. pullorum* was isolated. On the next test at 10 months of age, there were no reactors in this group, but at 1 year, 1 bird reacted with variant, but not regular, antigen. The ovary was markedly pathologic, but the organism was not recovered on culture. Another bird showed a trace of reaction at this time with variant antigen, but there was no evidence of infection on autopsy or bacteriologic examination. The group remained negative up to the time of disposal some six months later. Three lots of eggs were hatched from the negative and three lots from the positive group. Eleven per cent of the chicks from the negative group died with no recoveries of the organism, while 73 per cent of the positive group died, and *S. pullorum* was recovered from 90 per cent of them.

Mixed Antigens.—In the course of this work, it became clear that neither regular nor variant antigen alone would prove satisfactory for detecting the heterologous infection. A whole blood antigen composed of equal volumes of regular and variant antigen was prepared and gave good results in our controlled testing.[5] The whole blood test with regular strain antigen detected more of the variant infected birds than did the tube method using a regular antigen. This may have been due to the greater concentration of serum in the former test. Whole blood and tube tests on variant infected birds showed an agreement of 96.5 and 97.3 per cent on two groups of 866 and 570 birds, respectively. Comparison of variant, regular, and mixed antigens on 1,391 birds, 746 of which were in variant-infected and 570 in regular-infected groups, showed an agreement of 99.4 per cent between the variant and mixed whole blood results, and 98.6 per cent between the regular and mixed whole blood tests, or 98.2 per cent on the 1,391 samples. In arriving at the percentage of agreement, complete and partial reactions were considered as positive, as is done in practice. The antigen was further tested in the field on several thousand birds, with satisfactory results, before consideration was given to using it in place of the regular antigen.

Cross Agglutination of Younie Types with Proteus.—Among cultures received from Dr. Hancock of Nova Scotia, there was a strain of Proteus isolated from a hen which had reacted to variant, but not regular, antigen. This organism produced acid and gas in galactose and glucose, acid in sucrose and levulose, and did not ferment lactose, maltose,

TABLE I—Differentiation of Types by Tube Test

Antigen	Variant serum 1:25	1:50	1:100	Regular serum 1:25	1:50	1:100	Proteus serum 1:25	1:50	1:100	Negative serum 1:25	1:50	1:100	Type
No. 1	1	0	0	3	4	4	0	0	0	0	0	0	R
2	4	4	4	1	3	3	2	4	4	0	0	0	V
3	0	0	0	1	4	4	0	0	0	0	0	0	R
4	2	1	1	3	4	4	0	0	0	0	0	0	R
5	3	2	1	4	4	4	0	0	0	0	0	0	R
6	1	0	0	3	3	4	0	0	0	0	0	0	R
7	3	3	2	4	4	4	0	0	0	0	0	0	R
8	4	4	4	2	4	4	4	4	4	0	0	0	V
9	3	3	2	2	4	4	0	0	0	0	0	0	R
10	2	4	4	0	0	0	2	3	3	0	0	0	V
11	0	0	0	4	4	4	0	0	0	0	0	0	R
12	3	2	1	1	3	4	0	0	0	0	0	0	R
13	0	0	0	2	4	4	0	0	0	0	0	0	R
14	3	3	1	4	4	4	0	0	0	0	0	0	R
15	0	0	0	4	4	4	0	0	0	0	0	0	R
16	0	0	0	2	4	4	0	0	0	0	0	0	R
Variant	4	4	0	4	4	4	4	4	4	0	0	0	V
Regular	1	0	0	4	4	4	0	0	0	0	0	0	R
Proteus	0	0	0	0	0	0	4	4	4	0	0	0	

mannitol, and dextrin in fourteen days. It produced indole and H_2S and liquefied gelatin rapidly. It rendered litmus milk alkaline and peptonized it. It was motile and grew out in a thin film from the line of inoculation. It was agglutinated to a varying degree by variant, but not by regular, serum. The most striking effect was the ability of Proteus antiserum to agglutinate variant, but not regular, strains of *S. pullorum*. A horse was given a series of injections of this strain of Proteus, and serum was collected. The horse was negative with both types of *S. pullorum* and Proteus before injection.

A critical examination of 136 strains of *S. pullorum*, was made with regular, variant, and Proteus serum. Thirty-eight were clearly established as Younie type by the use of the first two serums. All of these were agglutinated by Proteus serum in dilutions practically as high as Proteus antigen. Ninety-eight strains were shown to be of the regular type. None of these were agglutinated beyond a trace in 1 :10 with Proteus serum, and the majority were entirely negative.

The clear-cut agglutination of Younie types of *S. pullorum* by Proteus serum is definite evidence of the antigenic difference between the strains. It has been repeated many times on some subcultures, and the results are constant. It would, therefore, appear that not only is the agglutination an indication of an antigenic difference but, if examination of a larger number of cultures gives the same results, it would be a useful method of differentiation between the two types. The titer of the serum when first collected was 1 : 5,120 with Proteus and 1 : 1,280 with variant antigen. Regular antigen only showed a partial agglutination in 1 : 10. An example of the differentiation of the two types in one lot of 16 cultures is given in table 1.

The slide method appears to be equally satisfactory. Variant and regular serums are diluted in watch glasses to the point where they clearly differentiate the controls before the unknowns are tested.

TABLE 2—Absorbed and Unabsorbed Variant Serum

Serum	Antigens	
	Variant	Regular
Variant—absorbed	431000	000000
Regular—absorbed	431000	000000
Not absorbed	444431	444210

Dilutions of 1:20 to 1:640. Readings made at twenty-four hours. 4=complete agglutination. 0=no agglutination. 3 to 1=Intermediate degrees of agglutination.

Absorption Tests.—The following experiments are representative of the results obtained by absorption of variant, regular, and Proteus serum by strains of *S. pullorum* and Proteus. The method used was that outlined in the manual of methods of Pure Culture Study of Bacteria,[9] except that dilutions of 1 : 10 were usually employed, so that final dilutions of 1 : 20 upward might be made.

A variant type serum was absorbed with regular and variant strains. Four agar slopes of culture were used for each 10 cc. Two slants were added to the serum and incubated for two hours at 37 C. The serum was centrifuged and two more slants added, following which it was incubated for two hours and held in the refrigerator overnight. One portion was left untreated but was held under the same conditions as the others. Results of agglutination test with the absorbed and unabsorbed serums are shown in table 2.

It will be seen from table 2 that agglutinins were completely absorbed for regular strain antigen in the test dilutions by both regular and variant cultures, but neither of these removed the agglutinins for the variant antigen although they were reduced by both.

Results of the absorption of a regular type serum of low titer with variant and regular culture are shown in table 3.

TABLE 3—Absorbed and Unabsorbed Regular Serum

Serum	Antigens	
	Variant	Regular
Variant—absorbed	200000	000000
Regular—absorbed	100000	000000
Unabsorbed	432000	420000

Dilutions and readings as in table 1.

Agglutinins in this serum were removed for regular antigen and lowered for variant antigen by both variant and regular strains. The titer of the unabsorbed serum was lower with regular than with variant antigen, but the difference did not appear to be sufficient to account for the results. This serum was classified as being regular because it was produced by injection of a regular strain. It was collected four days after injection and at that time, as is frequently the case, had a higher titer for variant than for regular antigen.

An experiment on the absorption of Proteus serum with *S. pullorum* and Proteus is shown in table 4.

It will be seen from table 4 that the variant type absorbed practically all the agglutinins against itself in the Proteus serum, but did not remove the trace of agglutinins for regular antigen. The regular strain removed the trace of regular agglutinins, slightly lowered the variant agglutinins, but had no appreciable effect on the Proteus agglutinins.

Absorption of a serum, produced against the regular type of *S. pullorum*, with variant and regular strains and Proteus, is shown in table 5.

As will be seen in table 5, absorption of this regular type serum by variant culture removed practically all the variant, most of the regular, and the small amount of Proteus agglutinins that had been present. Absorption with regular culture removed all agglutinins for variant, regular, and Proteus. Absorption with Proteus removed most of the variant agglutinins, very little of the regular,

and all the Proteus agglutinins, in the dilutions employed. Two other regular type serums, 1 chicken and 1 rabbit, were absorbed with large quantities of Proteus. In both cases, the variant and regular agglutinins were reduced.

A variant serum was absorbed with the homologous strain, with regular type, and with Proteus, in the same manner as that shown in table 5. Results of this are given in table 6.

TABLE 4—Absorbed and Unabsorbed Proteus Serum

Serum	Antigens		
	Variant	Regular	Proteus
Variant—absorbed	110000	100000	111244
Regular—absorbed	124443	000000	123444
Proteus—absorbed	100000	100000	100000
Unabsorbed	244444	100000	244444

Dilutions and readings as in table 1.

It will be seen from table 6 that absorption of this variant serum with variant antigen removed all agglutinins for variant, regular, and Proteus. Absorption with regular culture removed regular and Proteus and greatly reduced variant agglutinins. Absorption with Proteus had no effect on variant or regular agglutinins but removed those for Proteus. Further Proteus culture was then added. The agglutinins against variant and regular were slightly reduced by this further treatment but were not removed.

From these and other absorption experiments, it appeared that regular agglutinins were more readily removed by either regular or variant strains than were variant agglutinins. In low titer serum, both types were removed by massive treatments with either regular or variant cultures. Regular agglutinins were but little affected by absorption with Proteus, whereas variant agglutinins were generally removed or lowered in titer. Exceptionally, as shown in table 6, variant agglutinins were only slightly reduced. The variant agglutinins in Proteus serum were removed by both Proteus and variant but only slightly lowered by regular

TABLE 5—Regular Serum Absorbed with Variant, Regular, and Proteus

Serum	Antigens		
	Variant	Regular	Proteus
Variant—absorbed	10000000	32110000	00000000
Regular—absorbed	00000000	00000000	00000000
Proteus—absorbed	41111000	44443200	00000000
Unabsorbed	44444410	44444421	31000000

Serial dilutions of 1:20 to 1:10,240 were set up. They are only shown up to 1:2,560.

strains. The Proteus agglutinins were removed by Proteus but were very little affected by variant or regular strains.

Incidence and Stability of Types.—In 1942, Glover and Connell,[4] using absorbed and natural serum, found that 110 of 161 isolations were of the variant type (68.3%). Whether or not due to the use of variant antigen for the past few years, only 20 of 88 strains recently examined by us were variant (22.7%). The Younie type has been identified among strains received from Nova Scotia, Quebec, Ontario, and Manitoba. Cultures from other provinces have not been examined at this laboratory.

While the cultures examined appear to be fairly well fixed, and do not appear to have been modified by animal passage, it does not necessarily mean that changes do not occur *in vitro*, nor that results would be the same *in vivo*. It is too soon, on the basis of our present information, to form an opinion on the development of these types.

Practical Applications.—The mixed whole blood antigen was introduced for use under Dominion Department of Agriculture policies in the fall of 1945. Some 3,500,000 doses have been used in those provinces where this method of testing is carried on. Difficulties were encountered in some flocks due to inherent qualities in the variant organisms which made the antigen more sensitive. These difficulties appear to have been overcome, largely through the wholehearted coöperation

TABLE 6—Absorption of Variant Serum with Variant and Regular Strains and Proteus

Serum	Antigen		
	Variant	Regular	Proteus
Variant—absorbed	0000000	0000000	0000000
Regular—absorbed	3100000	0000000	0000000
Proteus—absorbed	4444300	4440000	0000000
Unabsorbed	4444210	4444000	4200000

Serial dilutions 1:20 to 1:10,240 were set up. They are only shown up to 1:1,280 in this table.

of the testing authorities, and such information as we have received in connection with chick mortality indicates that the results have been very satisfactory. Where the tube method was practiced, the use of variant and regular tube antigen has been continued.

SUMMARY

An antigen prepared from regular strains of *Salmonella pullorum* did not detect all birds infected with the Younie type of this organism.

Homologous strains were successfully employed to remove birds infected with the Younie variant as shown by hatching and testing results.

A mixed, stained antigen showed a high degree of efficiency in detecting birds infected with both types.

A mixed, tube antigen was not as satisfactory, and the use of regular and variant antigens was continued for tube testing.

The Younie type of *S. pullorum* could only be distinguished by serologic methods. Bacteriologic methods and bacteriophage did not show any differential features.

Antiserum produced against a strain of Proteus isolated from a hen agglutinated the variant, but not regular, types in a series of 136 cultures examined. If confirmed by further work, this Proteus serum should be a useful method of differentiation.

Regular agglutinins were more readily removed by absorption with either regular or variant strains than were variant agglutinins.

Proteus tended to remove variant, but not regular, agglutinins, but this was not constant.

Variant agglutinins in Proteus serum were removed by both Proteus and variant, but only slightly lowered by regular strains.

Twenty of 88 strains recently isolated were of the Younie type (22.7 %).

The Younie type variant has been shown to be present in four provinces from which cultures were received for typing.

There has been no evidence of change in type after two years in our variant cultures, nor were they affected by animal passage.

Approximately 3,500,000 doses of mixed, stained antigen were used for whole blood testing in the past season, and reports so far indicate that results were good.

DISCUSSION*

DR. VAN ROEKEL.—The work of Dr. Gwatkin is significant. This subject was discussed at our last conference. Dr. Wright of Ontario examined a number of strains a year ago at the various laboratories at the Northeastern Conference, and he classified a number of these variant strains that were received from this country. Massachusetts had 12 strains, of which three were identified as variants and three as intermediates. Dr. Gwatkin didn't mention the intermediates, but this summer I sent Dr. Gwatkin 20 pullorum strains, including the three that Dr. Wright had previously classified as variants, but Dr. Gwatkin classified them as regular on his examination.

That might be explained by the fact that Dr. Gwatkin says they may vary a little—variant to regular, back and forth. However, I think it is important that we keep our eyes open in this matter and, as far as we can tell in our pullorum test work in Massachusetts, we haven't encountered any outbreaks where we couldn't eliminate the infection through testing.

I think considerably more work should be done on this typing. I am hoping Dr. Edwards will give us considerable help on this problem.

DR. GWATKIN.—I purposely avoided saying anything about Dr. Edwards, because he is going to bring that question up later in the year. I think it is permissible to say that we have good agreement. There may be some difference of opinion in interpretation.

That one strain that I found a variant with you, Dr. Van Roekel, was definitely agglutinated by the Proteus. There were some of the others, different perhaps, that showed a trace in 1 : 10. In view of the fact that the Proteus serum sometimes does that, I classed them as regular, and it may be there were variant colonies. I didn't examine them in detail. I think I examined two, looking at individual colonies. The others were examined in bulk. It is a big job to examine a hundred colonies in subculture.

*After the presentation of the paper, the following discussion was conducted.

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Cultivation of the Virus of Infectious Bronchitis of Chickens in Embryonated Chicken Eggs

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THE CULTIVATION and modification of the virus of infectious bronchitis of chickens in embryonated chicken eggs were reported by Beaudette[1] and by Delaplane.[2] The virus, when first isolated from chickens and inoculated into embryonated chicken eggs, failed to produce embryo mortality for the first few passages. With each succeeding passage, it became more virulent for embryos until finally the majority of them were killed by the end of the second day following inoculation. Its ability to kill embryos appeared to become so fixed that no further changes in this respect were noted. Cultivation in eggs attenuates the virus and alters the immunogenicity for chickens.

Studies of infectious bronchitis in chickens have been conducted at this institution for several years, and attention has been recently directed toward cultivation of embryo-adapted virus in an endeavor to obtain basic information which might be of assistance in future research problems. While the observations reported in this contribution may be somewhat miscellaneous in character, the results are submitted for the value they may have for other investigators.

PROCEDURE

A strain of bronchitis virus which had been adapted to cultivation in embryonated chicken eggs was used for inoculum.* This virus was capable of killing the majority of ten- or eleven-day embryos by the end of the second day and the remainder by the end of the fourth day. The virus suspension employed consisted of pooled allantoic fluid from embryos dead on the second day following inoculation with the virus *via* the allantoic sac route. The site for injection into the allantoic sac was determined by transillumination of the egg and selection of an area of the chorioallantoic membrane free of large blood vessels about 3 mm. below the base of the air cell. A short groove was drilled through the shell, without piercing the shell membrane, by means of a small carborundum disc on a mandrel attached to the chuck of an electric motor. Tincture of metaphen was painted over the groove in the shell and allowed to dry before the eggs were inoculated. After injecting the virus into the allantoic sac using a B-D Yale 1-cc. capacity tuberculin syringe fitted with a 27-gauge, 1/2-in. needle, the holes in the shells were sealed with melted paraffin and the eggs returned to the incubator. All incubation was at 99 F. in an electric, forced-draft incubator. The eggs were candled daily, and those with embryos dead on the second day were chilled at 4 C. for several hours, or in some cases overnight, before harvest. The shell over the air cell was cracked and removed with forceps and a 5- or 10-cc. capacity Luer syringe fitted with a 20-gauge, 1-1/2-in. needle was used to aspirate the allantoic fluid. Only clear allantoic fluid was harvested.

Virus was used in experiments on the effect of freezing and thawing of allantoic fluid, titration of embryo m.l.d. and diluents for titration, effect of amount of inoculum on titer and embryo mortality, and lyophilization.

FREEZING AND THAWING OF ALLANTOIC FLUID

Hirst, Rickard, and Whitman[3] reported that freezing and thawing of both normal and influenza-infected allantoic fluid yielded a fluffy, white precipitate soluble at 37 C., and that prolonged storage at —10 C. or 4 C. yielded an insoluble precipitate. The soluble precipitate consisted of both protein material and amorphous urates while the insoluble precipitate consisted mainly of urates and did not possess the properties of the soluble precipitate. The virus could be concentrated by centrifugation of thawed, infected fluid before the temperature of the fluid had risen appreciably, removal of the supernatant fluid, and resuspension of the precipitate to one-tenth volume using the supernatant fluid as the diluent. From 90 to 95 per cent of the virus was carried down with the sediment. The insolu-

Contribution No. 684 of the Rhode Island Agricultural Experiment Station, Kingston.

Presented before the Section on Poultry, Eighty-third Annual Meeting, American Veterinary Medical Association, Boston, Mass., Aug. 18-22, 1946.

Associate professor of bacteriology and public health, School of Veterinary Medicine, Michigan State College, East Lansing (Cunningham); and head, Department of Poultry Husbandry, Rhode Island State College, Kingston (Stuart).

*Virus originally supplied by Dr. E. F. Waller, University of New Hampshire, Durham, N. H. Present address: University of Vermont, Burlington.

ble precipitate and amorphous urates did not give any results in concentration of the virus by this means.

Stanley[4] evaluated several methods for the concentration and purification of influenza virus from allantoic fluid. Elution of the precipitate formed on freezing and thawing of allantoic fluid resulted in only a 50 to 90 per cent removal of chicken red blood cell agglutination activity from the supernatant fluid. Extracts of the precipitate contained over 80 per cent of nonspecific protein. High speed centrifugation was the most efficient means of concentration and purification of the virus.

Knight[5, 6] reported that the sedimentable protein of both normal and influenza-infected allantoic fluid is not appreciably affected by freezing and thawing and is readily soluble in M/10 phosphate buffer. These mutual properties provided no advantage in the purification of the virus.

When bronchitis-infected allantoic fluid was frozen and thawed, a considerable amount of yellow, aggregated precipitate insoluble at room temperature was present although the fluid before freezing was either transparent or translucent without any visible precipitate. Sedimentation of the insoluble precipitate by centrifugation yielded an almost water-clear, supernatant fluid. Refreezing and thawing of the decanted supernatant fluid produced only a small amount of insoluble precipitate.

In addition to the insoluble, yellow precipitate there was also a white precipitate soluble at room temperature. The soluble precipitate could be observed during the initial thawing of the fluid, but it was almost obscured by the insoluble precipitate and went into solution when the temperature of the fluid exceeded about 4 C. It was clearly visible during thawing of the refrozen, decanted, supernatant fluid.

Prolonged storage of the fluid at 4 C. produced an insoluble precipitate of small particle size in contrast to the aggregated precipitate from thawed fluid. Normal allantoic fluid was not appreciably affected by freezing and thawing.

The observations of the effect of freezing and thawing of bronchitis-infected allantoic fluid were similar to the reports of Hirst *et al.*[3], and of Stanley[4] with respect to the formation of precipitates. Bronchitis fluid differed from Hirst's description of influenza fluid in that the insoluble precipitate from bronchitis fluid could be observed during thawing of the sample and could be sedimented by centrifugation after being frozen for only a few hours. Prolonged storage in the frozen state was not required for formation of the precipitate.

In contrast to Knight's[6] report that the sedimentable protein of influenza-infected allantoic fluid was not appreciably affected by freezing and thawing, precipitates were formed when bronchitis-infected allantoic fluid was frozen and thawed. The insoluble precipitate from bronchitis fluid was not soluble in M/10 phosphate buffer at pH7.

During the early part of the work, freshly harvested, allantoic fluid, supernatant fluid thawed from —10 C. and clarified by centrifugation, or thawed fluid in which the insoluble precipitate had been resuspended usually contained 10^9 and, in some instances, 5×10^9 embryo m.l.d. per 0.2 cc. of inoculum. Later, when storage facilities at —25 C. and —70 C. were available, it was found that virus frozen at these temperatures, thawed, and clarified of the insoluble precipitate by centrifugation at about 3,000 r.p.m. for fifteen or twenty minutes yielded a supernatant fluid containing 10^8 and, in some instances, 5×10^8 or 10^9 embryo m.l.d. per 0.2 cc. of inoculum.

Attempts to concentrate the virus by sedimentation of the soluble precipitate as described by Hirst[3] and Stanley[4] for the influenza virus met with failure due to lack of facilities for refrigeration of the centrifuge. In several trials, the temperature of the fluid at the end of the centrifugation period was from 15 to 20 C. and the precipitate was in solution.

No explanation as to the reasons for the higher titers following freezing at —25 or —70 C. than at —10 C. is offered. Whether it was due to an increase of the virulence of the virus for embryos at some particular passage coincidental with freezing at the lower temperatures, or to a partial purification of the virus through removal of nonviral material by centrifugation, remains to be studied.

From 5 to 10 embryos were generally used for routine passage of the virus. The amount of allantoic fluid harvested varied from about 5 to 8 cc. per egg but, in some instances, as many as 10 cc. could be obtained. The harvest was pooled in 30-cc., screw cap vials (22 x 120 mm.) and frozen at —70 C. The fluid was thawed at room temperature, clarified by centrifugation, and the supernatant fluid distributed in several 5-cc. glass ampules which were sealed and stored at —70 C. If a large amount of inoculum was needed, a sufficient number of embryos were inoculated. The harvest was pooled in prescription bottles of suitable capacity and frozen at —70 C. The fluid was thawed at room temperature, the insoluble precipitate resuspended, samples placed in 30-cc. vials and processed as previously described.

TITRATION

In all titrations the titer was considered to be the highest dilution which killed 50 per cent or more of the embryos inoculated, and the numbers of embryo minimum lethal doses the reciprocal of the logarithm of the dilution. Eight eggs per dilution were used, as each tray in the incubator accommodated eight eggs per row. This was considered to be a significant number of eggs for accurate titration with the dilutions employed. Knight[7] reported that ten eggs are to be preferred to five eggs per dilution for most quantitative determinations of influenza virus activity.

Inoculum consisted of supernatant fluid from samples which had been frozen at —25 or

—70 C. and processed as described under "Freezing and Thawing of Allantoic Fluid."

One of the early problems was the development of a satisfactory technique for titration and the selection of the diluent. No significant differences could be observed between Difco nutrient broth, Difco tryptose phosphate broth, 0.85 per cent sodium chloride, or M/10 phosphate buffer (pH 7) when used as diluents in comparative titrations. Difco nutrient broth was selected as the diluent as a matter of convenience since a supply was maintained in the laboratory for bacteriologic purposes.

In the initial titration, serial tenfold dilutions of the fluid were made in the ratio of 1 part virus (0.2 cc.): 9 parts diluent (1.8 cc.) in tubes (12 x 100 mm.), using a separate pipette (1.0 cc., graduation interval 0.01 cc., to deliver to the tip) for each dilution which was aspirated into, and expelled from, the pipette 15 times. A 5×10^{-1} dilution was prepared in the ratio of 1 part of the 10^{-1} dilution (0.4 cc.): 4 parts diluent (1.6 cc.) from which serial tenfold dilutions were made. This procedure provided tenfold dilutions with intermediate steps and afforded a more accurate detection of virus activity than tenfold dilutions. Progressing from the highest to the lowest dilution, using one syringe for the entire operation, each of the eggs per dilution was injected with 0.2 cc. of inoculum. The mortality rates were too variable for selection of a dilution as the titer. These results suggested incomplete mixture of the virus with the diluent.

Attempts to refine the technique yielded a procedure which gave excellent results. Serial tenfold dilutions were made in the ratio of 1 part virus (0.5 cc.): 9 parts diluent (4.5 cc.) in 10-cc., screw cap vials (17 x 65 mm.). A 5×10^{-1} dilution was prepared in the ratio of 1 part of the 10^{-1} dilution (1.0 cc.): 4 parts diluent (4.0 cc.) from which serial tenfold dilutions were made. After mixing the virus and diluent with a pipette as previously described, the cap was replaced on the vial which was vigorously shaken 100 times. One syringe was used to inject the series of dilutions as described in the initial titration experiments. An example of a titration is as follows, in which the denominator represents the numbers of eggs inoculated and the numerator the numbers of embryos killed by the virus:

10^{-3}	5×10^{-3}	10^{-4}	5×10^{-4}	10^{-5}	5×10^{-5}	10^{-6}	5×10^{-6}
8/8	8/8	8/8	8/8	7/8	6/8	1/8	0/8

The titer of this sample would be the 5×10^{-5} dilution, and there would be considered to be 5×10^{5}, or 500,000 embryo m.l.d. per 0.2 cc. of virus.

EFFECT OF AMOUNT OF INOCULUM

Henle and Chambers[8] observed that higher titers of influenza virus were obtained when embryos were inoculated with low, rather than high, concentrations of inoculum. This work was confirmed by Miller.[10] Henle and Henle[9] reported that the low titers of virus which result from the use of high concentrations of inoculum can be attributed to the presence of inactive virus which exerts an inhibiting influence upon the propagation of the virus.

Beaudette[1] reported that the amount of inoculum was not responsible for death of embryos inoculated with bronchitis virus, and that embryos regularly tolerated inoculum of 0.5 cc. and 0.8 cc.

In routine cultivation of virus, 0.2 cc. per egg was used. Investigations as to the effect of the amount of inoculum on embryo mortality and titer were undertaken.

A sample of virus containing 10^{5} embryo m.l.d. per 0.2 cc. was injected into three lots each of 15, 10-day-old embryos in the amounts of 0.2, 0.1, and 0.05 cc., respectively. In the lots receiving 0.05 and 0.1 cc. per egg, 1 embryo each was dead on the first day following inoculation, and the remainder were dead on the second day. In the lot receiving 0.2 cc. per egg, 2 embryos were dead on the first day and the remainder on the second day. Fluid from embryos dead on the second day in each lot was pooled and frozen at —70 C. for the following periods before thawing for centrifugation and titration of the supernatant fluid: 0.2-cc. lot, one day; 0.1-cc. lot, two days; and the 0.05-cc. lot, three days. Titrations of each lot were made using 0.2 cc. of each dilution to afford a constant amount of inoculum for comparative purposes. In the following summary, the control is the original sample used for the experiment.

	10^{-3}	5×10^{-3}	10^{-4}	5×10^{-4}
Control	8/8	8/8	8/8	8/8
0.05-cc. lot	8/8	8/8	8/8	8/8
0.10-cc. lot	8/8	8/8	8/8	7/8
0.20-cc. lot	8/8	8/8	8/8	5/8

	10^{-5}	5×10^{-5}	10^{-6}	5×10^{-6}
Control	6/8	3/8	3/8	0/8
0.05-cc. lot	7/8	6/8	1/8	0/8
0.10-cc. lot	7/8	3/8	2/8	1/8
0.20-cc. lot	5/8	2/8	2/8	0/8

These data indicate that, under the conditions of the experiment, the inoculum of 0.05 cc. per egg produced a higher titer than the original sample of 0.2 cc. per egg, and that the titer produced by the 0.2-cc. and 0.1-cc. lots were approximately the same as the original sample.

As the result of these findings, 0.05 cc. was used as the inoculum for routine cultivation of the virus. Titrations of virus selected at random from subsequent passages showed an activity of 10^{5} embryo m.l.d. and, in some instances, 5×10^{5} and 10^{6} embryo m.l.d. The use of this small inoculum had the advantage of virus of high titer and a conservation of material and storage space in the dry ice refrigerator.

LYOPHILIZATION

Preliminary investigations were made of the effect of lyophilization on the virus. Virus containing 5×10^5 embryo m.l.d. per 0.2 cc. was distributed in 1-cc. amounts in 2-cc. capacity vials, shell-frozen in a dry ice-alcohol mixture, and dehydrated at 0.15 mm. mercury pressure for eight hours. The vials were sealed *in vacuo*, checked for leaks with a high frequency coil tester, and stored at 4 C. Seven days later, the virus was restored to volume with 1 cc. of sterile distilled water. Titration showed only 5×10^3 embryo m.l.d. per 0.2 cc or a hundredfold decrease of virus activity Previous experiments had shown that the shell freezing was without effect on the titer of the virus. The loss of virus activity was probably due to the dehydration process.

SUMMARY

Experiments were conducted on the effect of freezing and thawing of both normal and bronchitis-infected allantoic fluid, titration of embryo m.l.d. and diluents for titration, effect of amount of inoculum on embryo mortality and titer, and lyophilization.

Normal allantoic fluid was not appreciably affected by freezing and thawing.

Freezing and thawing of bronchitis-infected fluid produced two types of precipitate. One was soluble at room temperature, and the other was insoluble at room temperature. The insoluble precipitate could be sedimented by centrifugation leaving an almost water-clear, supernatant fluid. Subsequent freezing and thawing of the decanted supernatant fluid yielded only a slight amount of precipitate.

Supernatant fluid from samples frozen at —25 or —70 C. had a higher virus activity than supernatant fluid from samples frozen at —10 C. No explanation as to the reasons for the increased activity is offered. Whether it was due to an increase of the virulence of the virus for embryos at some particular passage coincidental with freezing at the lower temperatures, or to a partial purification of the virus through removal of nonvirus material by centrifugation remains to be studied.

No significant differences could be observed between Difco nutrient broth, Difco tryptose phosphate broth, 0.85 per cent sodium chloride, or M/10 phosphate buffer (pH 7) when used as diluents in comparative titrations.

Dilutions for titrations prepared in tubes by mixing the virus and diluents with pipettes yielded embryo mortality rates too variable for determination of the titer. Dilutions prepared in screw cap vials by mixing the virus and diluents with pipettes and then vigorously shaking the vials yielded satisfactory embryo mortality rates for determination of the titer.

Allantoic fluid harvested from embryos receiving 0.05 cc. per egg had a higher titer than fluid from embryos receiving 0.1 and 0.2 cc. per egg. The mortality rate for each lot was essentially the same.

Virus which had been dehydrated at 0.15 mm. mercury pressure for eight hours, sealed *in vacuo*, and stored at 4 C. for seven days had a hundredfold decrease of activity when restored to volume.

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Escherichia Coli in the Blood Stream of Adult Fowl Affected with the Ocular Form of Fowl Paralysis

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LITERATURE dealing with *Escherichia coli* is voluminous, but reports involving fowl are not numerous. A complete discussion of the particular strain here reported cannot be presented now. It was isolated in the course of some research on the transmission of fowl paralysis by direct blood transfusion.[1]

Hens visibly affected with fowl paralysis furnished the blood for the inoculation of 398 chicks. Of 37 such donor hens, 36 showed ocular lesions. Injections of 1 to 2 cc. of fresh, untreated blood were used intravenously, and from 1 to 58 chicks were inoculated from each donor. All chicks were observed daily for evidence of disease. Five donors furnished blood for injecting 85 chicks, of which 84 showed evidence of sickness in five days—wobbly gait, soiled feathers, and evidence of chilling. Yellowish, watery diarrhea developed, along with cyanosis of the beak and shanks, and was followed by an incipient paralysis which terminated in death in from one to ten days after the first symptoms. Hen 810 served as donor for 58 of the 84 chicks that died.

LESIONS

The lesions produced in the proventriculus by the organisms in the day-old chicks were rather constant and uniform, varying from slight to extensive hemorrhages and to ulceration of the mucous surface (fig. 1). In some cases, the hemorrhages and ulcerations covered 1/3 to 1/2 of the mucous surface of this organ. In some chicks, hemorrhages of the heart muscles and congestion of the spleen and liver were observed.

BACTERIOLOGY

An organism was readily recovered from the blood and organs of the affected chicks and from the blood of the 5 affected donor hens. Twenty, 1-day-old chicks were inoculated with a culture of this organism. Ten received 0.2 cc. of the inoculum and 10, 0.1 cc. subcutaneously. All of the chicks were dead at the end of the fourth day and, at autopsy, typical lesions were observed in the proventriculus. The rapidity with which the hemorrhagic ulceration occurred in the proventriculus might well be compared to its occurrence in the intestine of quail and chukar partridges affected with acute, ulcerative enteritis.

A culture of the recovered organism was submitted to Dr. P. R. Edwards, bacteriologist, University of Kentucky, who reported as follows:

> Upon biochemical examination, the culture was found to be a typical strain of *E. coli*. It produced indol, fermented lactose readily, failed to grow on citrate, and produced little or no H_2S. As yet, we have done no work on typing of colon cultures, nor do I know of anyone in this country who has.

At the suggestion of Dr. Edwards, a culture of this organism was submitted to Dr. G. Vahlne, Folkhalsan, Tomteboda, Stockholm, Sweden, the only person equipped to identify strains of *E. coli*. No report has been received.

In these transfusion experiments for the transmission of fowl paralysis, 5 (13.5%) of the fowl-paralysis-affected donor birds were carriers of an organism which, in the amount of blood used for the transfusions, was capable of causing the death of a high percentage of 1-day-old chicks. It should be pointed out, however, that the amount of blood transfused was probably

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Presented before the Section on Poultry, Eighty-third Annual Meeting, American Veterinary Medical Association, Boston, Mass., Aug. 18-22, 1946.

Professor (Durant); and assistant professor (McDougle); of veterinary science, University of Missouri, Columbia.

greater, comparatively, than any transfusion which has yet been attempted on bird, man, or other mammals, and that if a smaller amount of blood had been used the chicks receiving the blood from the 5 carriers might have escaped the disease. In more recent experiments involving visibly affected, fowl-paralysis hens of the same age, a test of the blood of 9 of 16 fowl failed to reveal the presence of *E. coli*. These 16 hens, however, came from one flock, whereas, the 37 involved in the other transmission experiment came from many flocks over the state and provided a greater opportunity for such infections to be contracted by the experimental fowl.

Further investigations should be made to determine the frequency and extent with which *E. coli* infection may occur in poultry flocks, since it has been demonstrated in the blood of adult hens affected with fowl paralysis. Investigators who contemplate testing the transmissibility of the virus of fowl paralysis by direct transfusion should first be certain that it is not present in the blood stream of the experimental donors.

DISCUSSION*

DR. A. ASCOLI (New Brunswick, N. J.).—What do you say about similar mobilization of Pasteurella? I saw something like that when we conducted experiments in cattle with virulent strains of tuberculosis. In a few cases, Pasteurella was mobilized. It seems that these bacteria were present in the body of animals which had recovered from pneumonia.

Do you think that the injection of virus without *Escherichia coli* does mobilize the bacteria?

DR. DURANT.—The injected animals in this case were day-old chicks. We assumed that they were healthy. We isolated the organism also from the 5 hens which furnished the blood for the transfusion. So, I would assume that the organism was in the blood of the donor hens.

DR. F. BEAUDETTE (New Brunswick, N. J.).—Didn't you culture this blood before it was given intravenously?

*After the presentation of the paper, the following discussion was conducted.

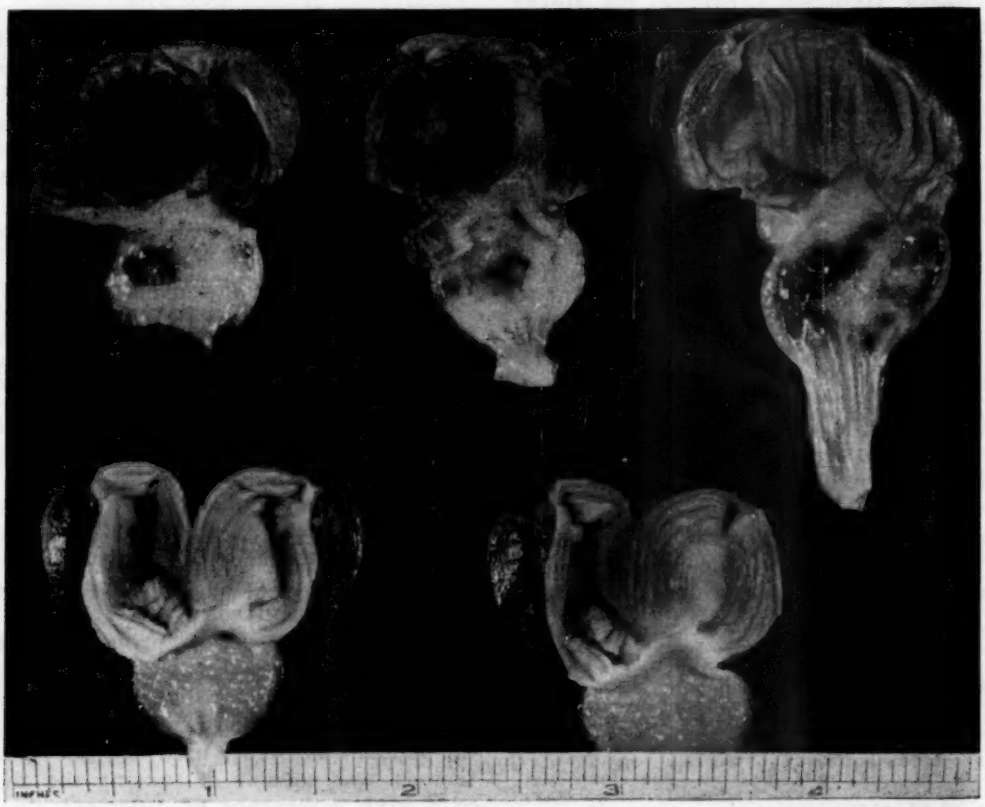

Fig. 1—The proventriculus and gizzards of 5 chicks. In the lower part of the picture are two healthy organs. The upper three represent varying degrees of hemorrhage and ulceration of the proventriculus. More than half of the mucous surface of the proventriculus in the upper right specimen is affected by hemorrhage and ulceration.

DR. DURANT.—No, but we still had the donor hen, so we drew more blood and cultured it.

DR. BEAUDETTE.—Didn't the hens which carried the organism as determined by injection later show it in the blood stream by direct culture?

DR. DURANT.—Yes, we isolated it.

DR. BEAUDETTE.—Were these birds moribund?

DR. DURANT.—No; like the other 37 hens, they looked healthy except for the ocular form of paralysis.

DR. BEAUDETTE.—Was the organism constantly free in the blood stream?

DR. DURANT.—We tested it only once. We isolated it from the day-old chick and then we drew blood from the donor hen and demonstrated that it was there. We isolated it several times from hen 810 after we got 58 fatalities in chicks inoculated from her blood. She seemed to carry it constantly, and it was easily recovered.

DR. BEAUDETTE.—How long did she live, or the others? Were they kept very long?

DR. DURANT.—About eight months. Some of them died at the end of six months, but most of them lived until the end of the experiment, which was eight months, and then we used all of them for autopsy.

DR. RONALD GWATKIN (Hull, Quebec).—We made some blood-agar plates from a rabbit some years ago and they were a terrible mess. I thought somebody had stumbled badly, rebled the rabbit, and again got a terrible mess. So we bled it the next day with the utmost care, made a microscopic slide and plated the blood. It was loaded with the coli-type organism. That rabbit lived between nine months and a year, apparently perfectly healthy, carrying a tremendous load of *E. coli*.

Also a good many years ago, Dr. Rumney brought in some hens with a history that suggested cholera. We opened the birds and found hemorrhages across the fatty band of the heart. I don't remember anything in the proventriculus. I was sure it was cholera and advised that we act accordingly. We cultured it to make sure, and the next day there was a heavy growth of coli. He wrote later that the losses had not been heavy enough to be an outbreak of cholera. They lost some 10 birds out of several hundred.

DR. MORRIS POVAR (Vineland, N. J.).—Did you notice any distinct change in the gizzard lining? I noticed on two of those lower slides the gizzard linings were much darker. Was that due to hemorrhage, or due to erosion?

DR. DURANT.—There was a little gizzard erosion, which is quite common in day-old chicks, but I think it had no connection whatever with the lesions seen in the proventriculus.

The Toxicity of Sulfamerazine to Chickens

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IN CONNECTION with the use of sulfaguanidine and other sulfonamide compounds in the treatment of coccidiosis of poultry and other livestock, it is essential to ascertain the effects of these drugs on the host. Swales[6] found that sulfamerazine given to chickens at rates of 2 or 3 Gm. per pound of feed "as bleeding began," reduced the mortality from cecal coccidiosis. Using normal rats, Endicott, Kornberg, and Daft[1] reported certain unfavorable effects from the administration of 1 per cent sulfamerazine in the feed. An examination of the bone marrow by these authors showed that there was a depletion of polymorphonuclear neutrophils, stab cells, and metamyelocytes; a marked increase in the number of nucleated red cells; gross hemorrhage in the subcutaneous tissues, retroperitoneal connective tissue, peritoneal, pleural and cranial cavities, and in the thymus, epididymis, testicle, gastrointestinal tract, kidney, eye, and nose. Welch *et al.*[7] fed sulfamerazine by capsule daily in amounts of 0.58 to 0.77 Gm. per kilogram of body weight for twelve days to 6 normal chickens averaging in weight 778 Gm. and found that 5 of the birds gained in weight; the weight of the sixth bird did not change. However, the sciatic nerve of 4 birds, the spinal cord of 1, and the kidneys of 2 showed evidence of injury. When a similar group of 6 birds were given 1.18 to 1.61 Gm. per kilogram of body weight, daily for twelve days, only 2 survived; of these 2 survivors, 1 suffered severe kidney damage. Severens, Roberts, and Card[5] reported that 2 per cent sulfamerazine mash was less effective than 0.5 per cent against pullorum disease of chickens. They suggested that the 2 per cent concentration of the drug might, therefore, be toxic. The final weights of the birds on the 2 per cent sulfamerazine diet were considerably less than those of the birds on the 0.5 per cent mash. McNeil and Hinshaw[3] administered 0.5 and 0.25 per cent sulfamerazine mash to turkeys infected with a respiratory disease. The birds receiving the higher percentage of drug ate normally for only four to five days, while the feed consumption of those on the lower percentage was normal for six to seven days. These authors believed that the 0.5 per cent sulfamerazine mash was unpalatable. Farr and Wehr[2] presented data to show that sulfamerazine, when administered for seven days in the mash in 0.25 and 1 per cent concentrations to noninfected and to Coccidium-infected birds, was somewhat injurious. An occasional bird showed spleen and/or liver lesions. The drug also interfered with the normal weight gains of the birds.

Mattis *et al.*[4] reported that no significant retardation in growth rate and no gross lesions resulted from the feeding daily of 0.5 to 1 per cent concentrations of sulfamerazine mash to 32-day-old chickens for two weeks.

In view of the conflicting results obtained by various investigators on the toxicity of sulfamerazine, further work was done by the authors of this paper to determine the effects of this drug on chickens of different ages.

MATERIALS AND METHODS

New Hampshire chickens from a commercial hatchery and Rhode Island Red chickens from the Animal Husbandry Division of the Bureau of Animal Industry at Beltsville, Md., were used in these tests. The birds were obtained as day-old chicks and were kept in metal brooders until used in tests. They were then transferred either into other metal brooders or into wire cages. The following ration was fed: corn meal, 25 per cent; pulverized oats, 15; wheat meal, 23.5; alfalfa meal, 6; soybean meal, 23.75; sardine meal, 3; limestone, 1.25; bonemeal, 1.5; manganized salt, 0.5; vitamin D (animal activated sterol), 0.037; butyl fermentation solubles (250 μg. riboflavin per Gm.), 0.462.

From the Zoölogical Division, Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C.

Sulfamerazine was administered in the mash or in gelatin capsules in amounts indicated under each experiment.

The postmortem examination of the birds for indications of toxicity was limited primarily to the detection of macroscopic lesions in such organs as the kidneys, heart, spleen, liver, and intestine; no histopathologic studies of any of the tissues, except the spleen, were made.

EXPERIMENTS AND RESULTS

Experiment 1.—Seventy-five Rhode Island Red chickens, 30 days old, were weighed and divided into five groups of 15 birds each. Groups 1, 2, 3, and 4 were fed 0.125, 0.25, 0.5, and 1.0 per cent sulfamerazine mash, respectively, for seven consecutive days. Group 5 served as a control. The birds were weighed twice a week throughout the test. Two weeks after withdrawal of the drug, all the birds were killed and examined *post mortem.*

During the week of medication, the birds of groups 1, 2, 3, and 4 made average gains of 117, 107, 72, and 49 Gm., respectively, while those of group 5 made an average gain of 125 Gm. For the following two weeks, groups 1, 2, 3, 4, and 5 made average gains of 188, 173, 182, 220, and 177 Gm., respectively. The total average weight gain for each group for the three-week period is shown in table 1.

Some of the birds of group 3 were badly injured by their cage mates, and 1 of them died as a result. No doubt, the injured birds felt indisposed to eat, thus accounting, at least in part, for the poor weight gains made by the birds of this group.

In this and in all subsequent tests, spleen lesions only were found in the treated birds. Ten of the 60 treated birds of this test had spleen lesions which varied from small hemorrhagic spots to caseous necrotic areas, 1 to 3 mm. in diameter. As may be seen in table 1, the distribution of birds with spleen lesions does not indicate that there was a very close correlation between amount of drug administered and presence of lesions.

Samples of tissue from a bird which had been fed 0.25 per cent sulfamerazine mash were examined by Dr. H. R. Seibold of the Pathological Division of the Bureau of Animal Industry. He reported that "the lesions in the spleen consisted of necrotic tissue surrounded by a capsule of histiocytes, giant cells, and fibroblasts. The microscopic picture suggests infarction."

Experiment 2.—Seventy, 16-day-old Rhode Island Red chickens were divided into five equal groups. Groups 1, 2, 3, and 4 received 0.25 0.5, 1.0, and 1.5 per cent sulfamerazine in the mash, respectively, for seven consecutive days; group 5 served as controls. During the first week of the test, the birds were weighed on the first, third, and sixth days. On the twelfth day of the test, 4 of the birds from each of the treated groups were killed and examined for lesions. The remaining birds were killed two weeks after withdrawal of medication.

During the first six days of the test, the members of groups 1, 2, 3, and 4 made average gains of 37, 27, 13, and 1 Gm., respectively; group 5, the controls, made an average gain of 44 Gm. For the following two weeks, the average gains of groups 1, 2, 3, 4, and 5 were 156, 124, 149, 154, and 127 Gm., respectively.

At necropsy, 7 of the 56 birds, including 1 of the group of 16 killed on the twelfth day, had lesions in the spleen. These lesions varied from a small hemorrhagic area—in 1 bird which had received 1.5 per cent sulfamerazine—to caseous, green, necrotic areas 1 to 5 mm. in diameter.

Experiment 3.—In tests 1 and 2, it was noted that the chickens fed a mash containing from 0.5 to 1.5 per cent sulfamerazine did not consume as much feed nor did they make as good gains as those receiving plain mash. The following test was devised and carried out to determine whether the drug produced these adverse conditions by exerting a toxic action on the chickens or by adversely affecting the palatability of the feed.

Seventy-two, 18-day-old New Hampshire chickens were divided into three groups of 24 each, the birds of each group being placed in a small brooder. At 9:00 a.m. on the day the experiment was begun, known quantities of feed and water were given to the birds of each group. Twenty-four hours later, the feed and water remaining in the containers were measured. By repeating this procedure throughout the experiment, daily records of the feed and water consumed were obtained. In addition to this, each chicken was weighed at approximately the same time each day. During the first seven days of the experiment, group 1 was fed unlimited amounts of plain mash, group 2 received unlimited amounts of mash containing 1.0 per cent sulfamerazine, and group 3 received amounts of plain mash each day equal to the amount of medicated mash consumed by group 2 on the preceding day. By following this procedure, group 3 actually entered the experiment one day later than the chickens in groups 1 and 2.

From the eighth to the thirteenth days, inclusive, all three groups were fed plain mash *ad libitum.* Throughout the entire experiment, all three groups received as much fresh water daily as they would drink. The chickens of all groups were killed and examined at autopsy two weeks after the start of medication.

The average daily weights of the chickens in the three groups from the second to the seventh day of the experiment are shown graphically in figure 1. The initial weights are omitted because they were taken at a time when all three groups had been receiving unlimited amounts of plain mash, whereas, the purpose of the experiment was to compare the weights of the groups

of chickens after they had become adjusted to their respective rations. Because of this procedure, group 1 was somewhat heavier than the other two groups at the first weighing shown on the graph, although at 18 days of age the greatest difference in average weights of the chickens in the three groups was only 0.7 Gm.

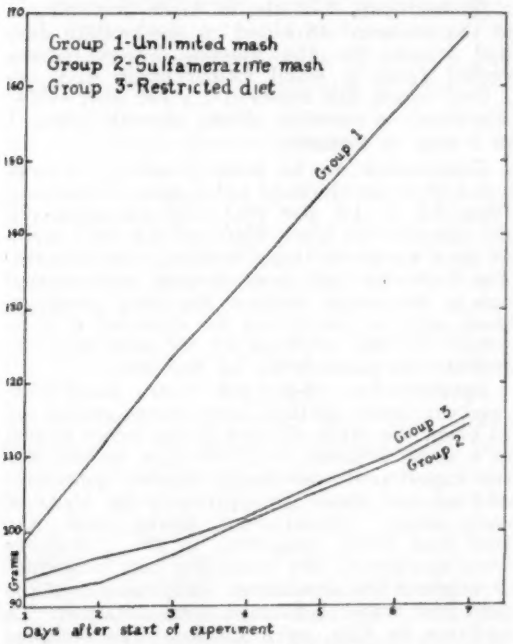

Fig. 1—Influence of 1 per cent sulfamerazine mash and of a restricted diet on the weight gains of chickens.

Group 1 chicks made consistently good gains each day, whereas, the birds in groups 2 and 3 showed a marked retardation in growth. The chicks in groups 1, 2, and 3 averaged 98, 92, and 93 Gm. in weight, respectively, at the beginning of the six-day period shown in the graph, and the average gain per chick during this period was 69 Gm. in group 1 and 23 Gm. in groups 2 and 3.

The average feed consumption per bird during the period covered by the graph was 144 Gm. for group 1 and 75 Gm. for groups 2 and 3. During this same period, the water consumption per bird was 299 cc. for group 1, 167 cc. for group 2, and 215 cc. for group 3.

From the eighth to the thirteenth day of the experiment, groups 1, 2, and 3 gained an average of 85, 79, and 108 Gm. per chicken, respectively. The average feed consumption per bird for this period was 191, 159, and 191 Gm., and the average water consumption per bird was 362, 304, and 377 cc. for group 1, 2, and 3, respectively.

It is apparent from the foregoing data that the retarded growth of the chickens in groups 2 and 3 is closely associated with a reduced feed consumption, since the chicks of these groups made comparable weight gains on identical amounts of feed. In group 3, the poorer weight gains resulted directly from the limited amount of mash, since group 1, which also received plain mash, but in unlimited amounts, made much greater weight gains than the birds of the former group. In group 2, which was given unlimited amounts of mash containing 1 per cent sulfamerazine, the question of the palatability of the medicated mash and the toxicity of the drug become factors in the lowered food intake and lesser weight gains of this group. As the methods of feeding of groups 1 and 2 differed only in the addition of sulfamerazine to the mash given group 2, both groups given unlimited amounts of their respective diets, the reason for the lighter birds of group 2 may be explained on the basis of unpalatability of the mash due to the presence of the sulfamerazine. However, group 2 made poorer gains than group 3 during the second part (8-13 days) of the experiment; this seems to indicate that the drug was toxic for the chickens in addition to being unpalatable.

No attempt was made to estimate the relative importance of the two aforementioned factors because it was not possible to estimate the influence of the age difference between the chicks of groups 1 and 2 and those in group 3. The fact that group 3 consumed its daily allotment of feed prior to 4:30 p.m. each day, instead of eating the food gradually over the 24-hour period as the other groups did, may also have had some influence on the weights of these birds.

At autopsy, no gross pathologic changes were noted in any of the chicks from groups 1 and 3. Each of 2 chicks from group 2 were found to have a single, yellow necrotic lesion about 1.5 mm. in diameter in the spleen; two, dark red hemorrhagic areas, each of which was about 1.5 mm.

across and of about the same depth were found in the spleen of another bird from this same group.

Experiment 4.—The evidence of the unpalatability of the medicated mash obtained in experiment 3 raised the question as to whether the poorer weight gains of the birds receiving the 1 per cent sulfamerazine might be due entirely to this factor or, to some extent, to the toxic effects of the drug. Experiment 4 was set up to throw some light on this question. Twenty-five, 43-day-old New Hampshire chickens were weighed and placed in three groups. For seven days, the 10 members of group 1 received 1 per cent sulfamerazine mash, and the 5 members of group 2 received a daily dose of 0.5 Gm. sulfamerazine in a gelatin capsule. The 10 birds of group 3 served as controls. A record was kept of the feed and water consumption of all groups, and the birds were weighed twice a week. One week after the withdrawal of medication, all the chickens were killed and autopsies held.

During the week they received the sulfamerazine, the average feed consumption of group 1 was 364 Gm. per bird; that of group 2 was 378 Gm. per bird; and that of group 3 was 585 Gm. per bird. During the week subsequent to administration of sulfamerazine, the average feed consumption per bird of group 1 was 584 Gm.; group 2, 579 Gm.; and group 3, 738 Gm. The water consumption in the three groups was in the same ratio as the feed consumption. The average weight gain during the first week for group 1 was 43 Gm.; group 2, 61 Gm.; and for group 3, 179 Gm. During the second week, the average gains for groups 1, 2, and 3 were 180, 167, and 175 Gm., respectively. There was little difference in the average amount of drug which the members of groups 1 and 2 received. The birds of group 2 were each given a total of 3.5 Gm. and, on the basis of average feed consumption per bird, the members of group 1 received 3.6 Gm. sulfamerazine. Eighty per cent of the 15 treated birds had lesions in the spleen. In 2 chickens of group 1, the lesions consisted of a few, dark, hemorrhagic spots while, in the remaining 6 birds, the lesions ranged from scattered, small, caseous spots to a condition where four-fifths of the spleen was replaced by green necrotic material. Four birds of group 2 exhibited scattered caseous spots throughout the spleen.

TABLE I—Effect of Administration of Sulfamerazine to Chickens for Seven Days

Exper. no.	Birds: Age (da.)	Birds: Breed	Birds: No./group	Birds: Av. wt./group (Gm.)	Administration sulfamerazine for 7 days	Av. wt. gains/bird (Gm.)	Birds with spleen lesions: No.	Birds with spleen lesions: %
1[1]	30	R.I.R.	15	229	0.125 % mash	305	0	0
				229	0.25% mash	280	2	13
				232	0.50% mash	254	6	40
				231	1.0% mash	269	2	13
				230	None (controls)	302	0	0
2[2]	16	R.I.R.	14	106	0.25% mash	193	1	7
				107	0.50% mash	151	1	7
				100	1.0% mash	162	3	22
				102	1.5% mash	155	2	14
				99	None (controls)	171	0	0
3[3]	18	N.H.	24	94	None (controls)	176	0	0
				93	1.0% mash	115	3	12
				94	None (restricted diet)	130	0	0
4[3]	43	N.H.	10	449	1.0% mash	223	8	80
			5	429	0.5-Gm. dose daily	228	4	80
			10	459	None (controls)	354	0	0

[1] Birds held for two weeks after termination of medication. [2] Four birds from each group killed on twelfth day of test; rest killed on twenty-first day. [3] Birds held for one week after termination of medication.

R.I.R.=Rhode Island Red. N.H.=New Hampshire.

The records of feed consumption of the two treated groups during the time the birds received the drug showed that those of group 2 consumed very little more than those of group 1 and that both groups consumed considerably less mash than the controls (group 3). Therefore, the drug itself must have had an adverse effect on the appetite; although the fact that the birds of group 2 received their daily allotment of sulfamerazine *via* capsules rather than in the mash may have accentuated this effect.

DISCUSSION

In reviewing the results of these four tests, it is apparent that there was a retardation in weight gains for all the groups fed the drug at levels of 0.5 per cent and above. In experiment 3, the birds were retarded in growth due to the

unpalatability of the 1 per cent sulfamerazine mash but, in experiment 4, the drug also was demonstrated to produce adverse effects on the appetite. As seen in table 1, spleen lesions were present in some members of all groups treated with sulfamerazine in concentrations higher than 0.125 per cent, but the incidence of the lesions was not very closely associated with the amount of drug administered. It is interesting to note that the 43-day-old birds of experiment 4 had a much higher percentage of spleen lesions than the younger birds of the other experiments.

SUMMARY AND CONCLUSIONS

1) One hundred sixteen Rhode Island Red and 63 New Hampshire chickens, 16 to 43 days old, received sulfamerazine in the mash or in 0.5-Gm. daily doses administered in capsules for one week to determine the effects of the drug.

2) All groups treated with sulfamerazine in the mash at levels higher than 0.25 per cent and the group given 0.5-Gm. doses daily made poorer weight gains than the controls.

3) This retardation in weight gain was due both to the unpalatability and to the toxic effects of the drug.

4) Spleen lesions were present in a few birds of all groups which received the medicated mash at levels of from 0.25 to 1.5 per cent and in 4 of the 5 birds which received 0.5-Gm. doses daily.

5) Birds 43 days old seemed to be more susceptible to splenic damage than birds 16 to 30 days old.

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